Sources and environmental controls of microbial membrane lipids in soils and groundwater

Dissertation (Kumulativ)

zur Erlangung des Doktorgrades

der Naturwissenschaften

- Dr. rer. nat. -

Vorgelegt dem Rat der Chemisch-Geowissenschaftlichen Fakultät

der Friedrich-Schiller-Universität Jena

Su Ding

Geboren am 12.02.1988 in China

Gutachter:

1. Prof. Dr. Gerd Gleixner

   Max Plank Institute for Biogeochemistry, Jena, Germany

2. Prof. Dr. Georg Pohnert

   Friedrich-Schiller-Universität, Jena, Germany

Tag der Verteidigung: 24.07.2019

Table of Contents

Table of contents

Acknowledgements

Gerd, thank you for your unconditional support, guidance and giving me the freedom and good resources to work on these exciting projects. As a supervisor, you are not only guiding me on the way of science, but also telling me the truth of life to help me to be a better man. Georg, every time I encountered the bottleneck of the experiment and felt depressed, you can always find a smart solution to refresh my mind and help me out of the woods. I will never forget those inspiring scientific discussions with you.

Valerie, thank you for letting me develop my own ideas and contributing significantly to my work. I'm deeply grateful for your great patience, endless support and for teaching me in the noble arts of liquid chromatography. Markus, thank you for sharing countless ideas, data and extensive discussions on the project. Without your consistent and illuminating guidance, my part of the jena experiment could not have reached its present form. I've really enjoyed working with you for the past 3 years.

I thank Susan for your continuous support from the start of my Ph.D. study. I would like to express my deeply gratitude to Prof. Kai-Uwe Hinrichs and his lab. I've really learned a lot and enjoyed the time that I spent my research stay in Bremen. Particular thanks to Julius Lipp who supported a great part of my work. You are so talented in mass spectrometry and liquid chromatography. Thank you for answering the countless questions and guiding me into a whole new world of membrane lipids. I am sincerely grateful to Prof. Ruth Welti and her group. Thank you for so many constructive comments and suggestions on the work. You provide me so many good opportunities to talk to the experts in different field, treating me like your own student and let me feel at home. I would also like to thank Mary Roth who shared her lipidomic experience with me.

Thank you, Vanessa, Franzi, Somak, Stefan, Carsten, Simon, Natalie, Kasun for sharing your ideas and discussions with me. I'm also appreciated for the help of Nico, Steffen, Uta, Robert, Ines, Heiko, Iris, Maria, Cassandre, Yinghui, Wenjie, Shangzhe, Xiu for your support of this work. I'm grateful for all the institute members that are not mentioned here for such a nice working atmosphere. I want to thank our secretaries Steffi, John and Kerstin. You are always there when I need your help.

My big thanks would go to our Chinese family, Haiyang, Yan, Jianbei, Yunpeng, Dapeng, Zhigang, Biao Huai, Dechang, Jingyuan, Feng Peng, you made me feel like home here.

Last but not least, thanks to all my friends and my family for their support of last three years. Chunjiang, thank you for your encouragement and accompany in this long way journey.

List of abbreviations

AEG	acyletherglycerol
APCI	atmospheric pressure chemical ionization
brGDGT	branched glycerol dialkyl glycerol tetraether
CDP	cytidine diphosphate
CL (cardiolipin)	diphosphatidylglycerol
Cren	crenarchaeol
Cren'	crenarchaeol regioisomer
DAG	diacylglycerol
DEG	dietherglycerol
DGDG	digalactosyldiacylglycerol
DGTS	diacylglycerylhydroxymethyltrimethyl-(N,N,N)-homoserine
DMPE	phosphatidyl-(N,N)-dimethylethanolamine
GC	gas chromatography
GDGT	glycerol dialkyl glycerol tetraether
GTGT	glycerol trialkyl glycerol tetraether
HILIC	hydrophilic interaction liquid chromatography
HPH	hexose-phosphohexose
HPLC	high performance liquid chromatography
IPL	intact polar lipid
isoGDGT	isoprenoid glycerol dialkyl glycerol tetraether
MGDG	monogalactosyldiacylglycerol
MMPE	phosphatidyl-(N)-methylethanolamine
m/z	mass to charge ratio
MS	mass spectrometer/spectrometry
NP	normal phase
OL	ornithine lipid

OM	organic matter
PA	phosphatidic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PI	phosphatidylinositol
PL	phospholipid
PS	phosphatidylserine
qToF-MS	quadrupole time-of-flight mass spectrometer
RI	ring index
RP	reverse phase
Sph	sphingolipid
SPM	suspended particle matter
TLE	total lipid extract
TOC	total organic carbon

1. Introduction

Lipids are essential components of microbial membranes (Op den Kamp, 1979). They play critical roles in microbial functions, including signal transportation, cellular barriers, energy repository and membrane architectures (Cronan, 1978; Cronan and Gelmann, 1975). Membrane lipids are very complex and highly dynamic, there are hundreds of molecular species and they are always changing with physiological and environmental changes (Dowhan, 1997; Nikaido and Vaara, 1985; Woese, 1987). It is not clear why nature created so diverse types of lipids. Recently, lipidomics, a novel discipline has emerged to study membrane lipids based on analytical chemical tools, particularly mass spectrometry (Brügger, 2014; Han and Gross, 2003, 2005; Yang and Han, 2016). Lipidomics can help us to identify new lipid molecules, reveal the mechanisms underlying responsible for physiological or environmental conditions, discover potential biomarkers to understand climate change, organism interaction and evolution (Han et al., 2012; Shevchenko and Simons, 2010; Wenk, 2005). Besides, stable isotope probing of certain lipid types reveals the activity and function of distinct microbial community in the environment (Wegener et al., 2016). Accordingly, with lipidomics, our understanding of the microorganisms in the complex ecosystem will be undoubtedly accelerated.

In this introduction, first I briefly introduced the classification of life and the importance of microorganisms in the environments. Then I summarized the tools to study microorganisms and compared them to the mass spectrometry-based lipidomic techniques. In addition, the type and function of archaeal and bacterial membrane lipids were introduced for better understanding lipidomic technics. Finally, I discussed the advantages and limitations of recently used lipidomics derived biomarkers and highlighted the aim of this thesis.

1.1 Tree of life

Based on the biological classification of Woese et al. (1990), life on the planet can be divided into three different domains – archaea, bacteria and eukaryote (which consists of plants, animals, fungi and protists, Fig. 1.1). The reason why prokaryotes are separated into two groups, archaea and bacteria, is that neither of them are precursors of each other. Besides, there are significant structural and genetic differences between them (Woese and Fox, 1977). Archaea contain genes and proteins that are more homogenous to Eukarya than Bacteria (Auer et al., 1989; Kimura et al., 1989).

Bacteria and archaea live almost everywhere on Earth. Particular archaea were initially thought as extremophiles thriving in harsh environments (e.g., hot spring or hypersaline water) because of their distinct physiologies (Kvist et al., 2007; Narasingarao et al., 2012). But they have been ubiquitously found in soils, peats, lakes and seas (Auguet and Casamayor, 2008; Leininger et al., 2006; Turich et al., 2007; Weijers et al., 2006a). In addition, archaea constituted a large percentage of component of marine plankton (Ouverney and Fuhrman, 2000), which was seen as one of the most abundant organism groups on Earth.

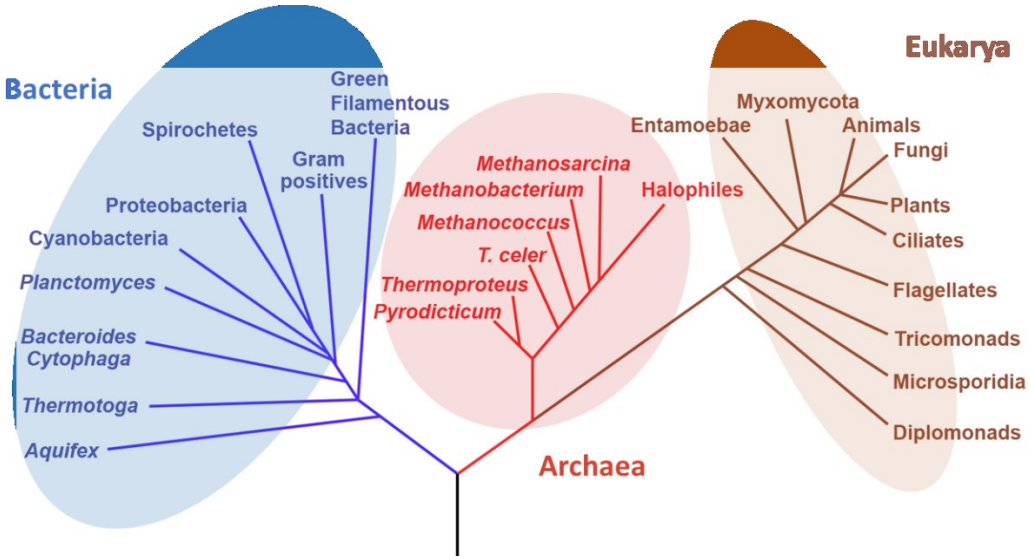


Fig. 1.1. The three-domain system based on 16s rRNA gene data, adapted from Woese et al. (1990). The Bacteria is colored in blue, the Archaea in red and the Eukarya in brown.

1.2 Biogeochemical importance of microorganisms in the environment

Microorganisms play important roles in biogeochemical cycles, soil formation and fertility, and decomposition of organic matters (Azam et al., 1983; Gadd, 2010; Handelsman et al., 1998). Various metabolic activities of microorganisms guarantee them to catalyze most chemical reactions in natures (Handelsman et al., 1998). The effect of microorganisms on the ecosystem can be beneficial or harmful (Stouthamer and Luck, 1993). Soil microorganisms, even the pathogens, are significant regulators of plant diversity and plant community dynamics (Van Der Heijden et al., 2007).

The most significant effect of microorganisms on earth may be their ability to recycle carbon and nitrogen that make up all living life (Alongi, 1994; Altieri, 1999). Atmospheric nitrogen could not be used by most eukaryotes. However, nitrogen is necessary for plants and animals to create proteins and nucleic acids for their growth (Wetherell and Dougall, 1976). There are some microorganisms called nitrogen fixing bacteria that can convert atmospheric nitrogen to ammonia for the use of plants and animals (Bohlool et al., 1992; Fischer, 1994; Madigan, 1995). Some bacteria fix N_2 in symbiotic associations with plants (Long, 1989). Other N-fixing bacteria are free-living ones present in soils and water (Vadakattu and Paterson, 2006). For example, symbiotic Nitrogen fixer *Rhizobia* which living in the nodules of legume helps legume to fix nitrogen for survive (Zahran, 1999). There are also a number of small groups that can contribute to ammonia oxidation in the environments, ammonia-oxidizing bacteria and ammonia-oxidizing archaea (Francis et al., 2005; He et al., 2007). In addition, some bacteria are primary producers, like cyanobacteria, they can perform oxygenic photosynthesis a variety of environments (Hamilton et al., 2016).

1.3 Tools to study microorganisms

There are various tools and techniques to study microorganisms in the environment. For measuring the size of microbial populations, the microscopic method could be used. It allowed microorganisms to be stained variously and counted directly (Babiuk and Paul, 1970; Schmidt and Paul, 1982). However, it took very long time and often met problems in distinguishing living cells from dead ones. Chloroform fumigation-incubation method (CFI) and the substrate-induced respiration method (SIR) are both rapid and objective methods for estimating the amount of living microbial biomass (Anderson and Domsch, 1978; Tateishi et al., 1989). However, they also have their limitations. For instance, CFI was confused by the difficulty in determining the contribution of non-microbial carbons to the fumigation (Horton et al., 1987). SIR method may overestimate biomass because the use of glucose may hinder the metabolism of other microbes (Martens, 1995).

To study microorganisms, microbiologists also applied pure cultures in the laboratory. Research of microbial cultures have helped us understand their physiological and pathological properties (Janssen et al., 2002; Kaeberlein et al., 2002; Rappé et al., 2002; Zengler et al., 2002). However, it has been proven that over 95% of microbes are not culturable (Amann et al., 1995; Fenchel and Ramsing, 1992; Torsvik et al., 1990). Microbes grown in the laboratory might differ from their natural counterparts in crucial ways. Short period cultures in laboratory conditions might cause physiological adaptation and thus change the way of growth. Moreover, pure cultured microbes may lack the ecological interactions that may exist in natural communities.

At present, there are two main methods that can overcome the problem of culturing bias. One is ribosomal RNA sequencing approach, the other one is lipidomic approach. Sequencing based studies provide a direct view of occurring microbial communities by identifying their phylogeny and taxonomic diversity (Flynn et al., 2013; Griebler and

Lueders, 2009; Madsen, 2000; Zhou et al., 2012). It allows us to understand microbial gene expression and cell metabolism from communities in the nature regardless possibility to culture them in the laboratory (Barns et al., 2007; Chan et al., 2006). Nevertheless, they do not provide detailed information about their community structure and functional groups, e.g., the biomass of dominant archaea and bacteria (Green and Scow, 2000; Lipp and Hinrichs, 2009; Martino et al., 1998; Schippers et al., 2005). Lipid-based biochemical techniques can effectively compensate for the shortcomings of molecular markers. For example, phospholipid fatty acid (PLFA) was considered as a useful tool to evaluate total living biomass, physiological and functional diversities of entire bacterial communities (e.g., Green and Scow, 2000; Jia and Conrad, 2009; Rooney-Varga et al., 1999; Schwab et al., 2016). Another important membrane lipid method, glycerol dialkyl glycerol tetraethers (GDGTs), could be used to assess the archaea community in the environment (Schouten et al., 2002; Sinninghe Damsté et al., 2002).

1.3.1 Microbial membrane lipids – diversity in structures and functions

As the major component of membrane, membrane-building lipids are made up of a core lipid, generally a hydrophobic alkyl chain ester- or ether- linked to a glycerol backbone and a polar head group (Fig. 1.2). These membrane lipids compose a monolayer or bilayer cell envelope that acting as a permeability barrier to water and ions (Krishnamoorthy et al., 2016). They protect microbes from unpredictable hostile environment, allow selection of necessary nutrients outside of the cell wall and discard the waste from inside (Silhavy et al., 2010).

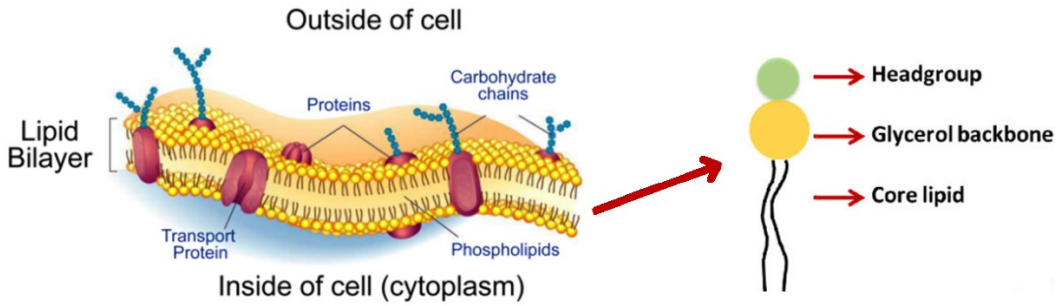


Fig. 1.2. Structure of cell membrane. The figure is based on a publicly available image, reproduced by Shadiac et al. (2013) and modified here.

Bacterial membrane lipids. Like eukaryotes, most bacterial membrane lipids are made up of two fatty acids esterified to glycerol (diacylglycerol) and a hydrophilic polar head group (Fig. 1.2, Epand and Epand, 2009). These diacylglycerols always consist of variable carbon number of chain length and unsaturation degrees (Epand and Epand, 2010). Sometimes their chains also contain zero to two branched methyl groups and up to one cyclopropane ring (Cronan, 2003). Besides, acyletherglycerol and dietherglycerol are common among anaerobic and microaerophilic bacteria (Pearson, 2014; Rethemeyer et al., 2010; Schubotz et al., 2009).

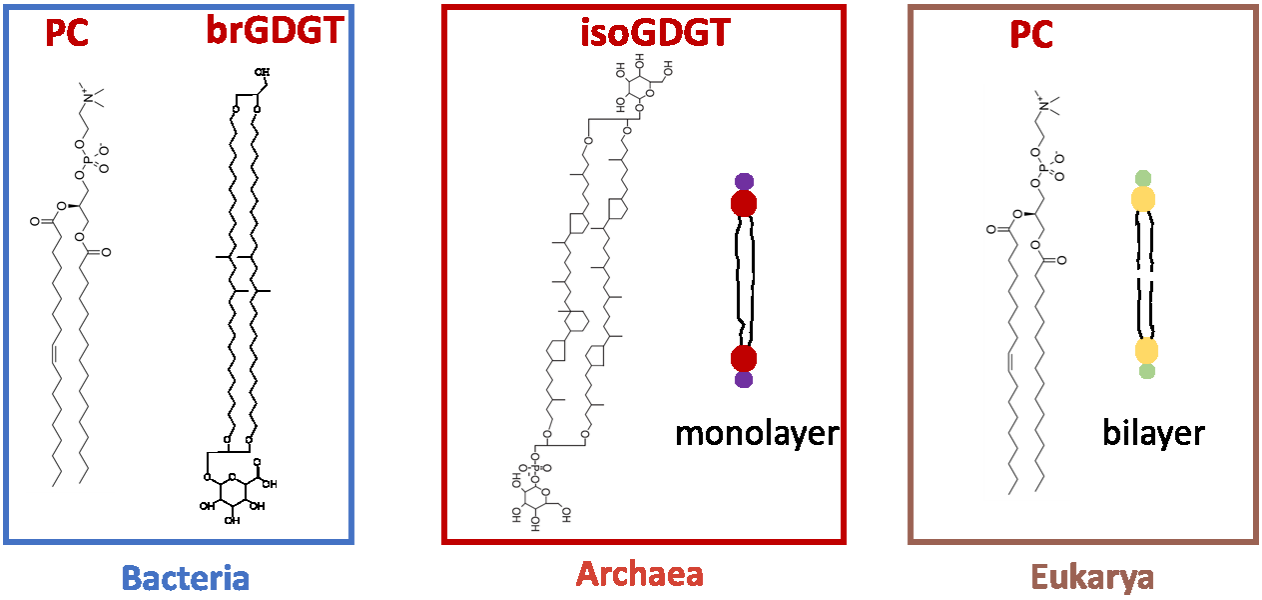


Fig. 1.3. Cell membrane lipids of three different domains (Bacteria, Archaea and Eukarya).

There are several phosphate head groups, phosphatidylethanolamine (PE), phosphatidylglycerol (PG), cardiolipin (CL), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylserine (PS) and lysophospholipids (lysoPL, Fig. 1.3). Bacteria can also produce phosphorus-free lipids such as diacylglycerylhydroxymethyltrimethyl-(N,N,N)-homoserine (DGTS), ornithine lipids (OL), monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfolipids (Geiger et al., 2010a; Geiger et al., 2010b; Sohlenkamp and Geiger, 2016; Vences-Guzmán et al., 2012). Bacteria has a complex pathway to biosynthesis these two types of membrane lipids and both of them are metabolically connected (Fig. 1.4, Sohlenkamp et al., 2003).

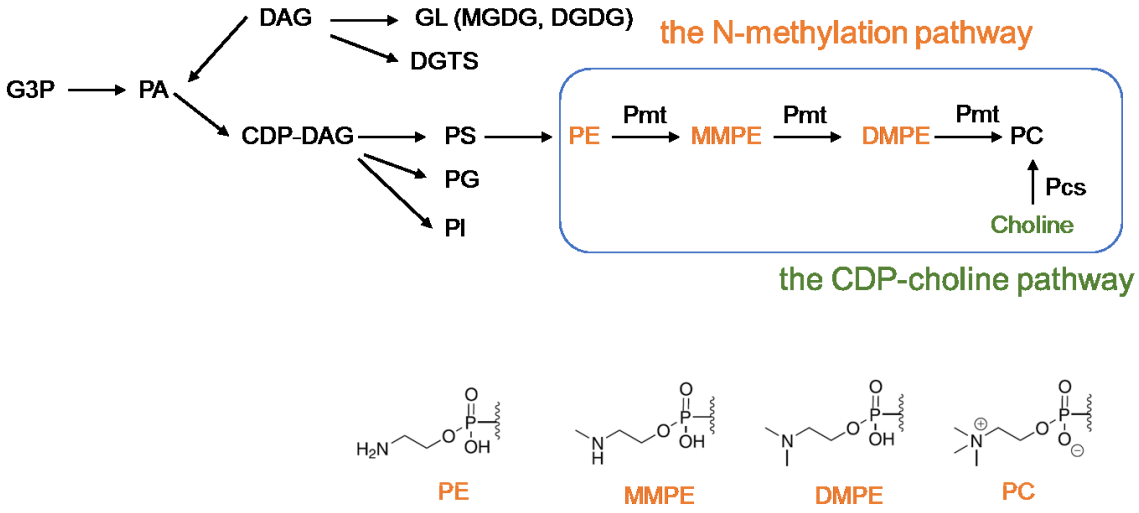


Fig. 1.4. Major metabolic pathways of membrane lipids in bacteria simplified from Sohlenkamp and Geiger (2016). Abbreviations: G3P-glycerol-3-phosphate; PA-phosphatidic acid; DAG-diacylglycerol; CDP-DAG-cytidine diphosphate-diacylglycerol; PS-phosphatidylserine; PE-phosphatidylethanolamine; MMPE-

phosphatidyl-(N)-methylethanolamine; DMPE- phosphatidyl-(N,N)-dimethylethanolamine; PC-phosphatidylcholine; PG-phosphatidylglycerol; CL-cardiolipin; LCL-lysyl-cardiolipin; PI-phosphatidylinositol; DGTS-diacylglyceryl-N,N,N-trimethylhomoserine; Pmt and Pcs are enzymes been used for the formation of phosphatidylcholine.

The common precursor cytidine diphosphate-diacylglycerol (CDP-DAG) is first to be synthesized by glycerol-3-phosphate and then form the other membrane lipids (Fig. 1.4). For example, PE is a most common membrane lipid in bacteria. CDP-DAG first react with serine to form anionic lipid PS. PS is then decarboxylated to synthesize the zwitterionic lipid PE (DeChavigny et al., 1991). The formation of PC could be achieved in two pathways. One is Kennedy pathway in which bacteria use choline from their host to directly form PC (López-Lara and Geiger, 2001). The other way is N-methylation pathway, PE is N-methylated three times to the final product PC (López-Lara et al., 2003; Sohlenkamp et al., 2003). Functions of phospholipids in the environment are very different. Some of them play important role as bridge connecting bacteria with eukaryotes. For example, *Sinorhizobium meliloti* can form PC using choline obtained from an associated plant. Mutants lacking PC were unable to form nodules on their host and thus their host had with serious growth defects (López-Lara et al., 2003). Some have a function of membrane structure, i.e., PC, PE and PG. Many soil bacteria restructure their membrane lipid composition in response to the stress from the environment (López-Lara et al., 2005). For instance, the phospholipids PC and PE can be replaced by phosphorus-free membrane lipids DGTS, GL and OL in response to the conditions of phosphorus limitation (Geiger et al., 2010a; Geiger et al., 2010b; Geske et al., 2013). With growth temperature increasing, bacterial membrane fluidity increases. Bacteria respond to increased temperature by increasing their fatty acid chain length or decreasing the degree of unsaturation in their membrane lipids (Zhang and Rock, 2008). Generally, plants have no odd number chain acyl species in their lipids (Pereyra et al., 2006; Wang et al.,

2006). In cultured bacteria, gram-positive ones have abundant PG and PE, in which the acyl chains are mainly odd-numbered branched-chain fatty acids. Gram-negative ones contain PE with even-numbered straight chain and cyclopropane acyl chains (Romantsov et al., 2009).

Another type of bacterial membrane lipid is branched GDGTs (brGDGTs) which contain two C_{28} liner alkyl chains linked by the ether bond to glycerol and head groups (Sinninghe Damsté et al., 2000). Until now, only some Acidobacteria were identified as producers of brGDGTs (Sinninghe Damsté et al., 2011), but the ubiquitous occurrence of brGDGTs in soils/peats, lakes and marginal seas suggests that there are other unknown biological sources (Peterse et al., 2011; Sinninghe Damsté et al., 2011; Weijers et al., 2009). These monolayer lipids differ both in the number of branched methyl groups and cyclopententyl moieties in their alkyl chains (Sinninghe Damsté et al., 2000; Weijers et al., 2006b). Like the bacteria produce bilayer diacylglycerol, brGDGT-producing bacteria also change their structures in order to carry out the cell function properly when the environment changes (Weijers et al., 2007). For example, bacteria add the methyl groups on the alkyl chains to changing growth temperatures (De Jonge et al., 2014; Peterse et al., 2012; Weijers et al., 2007). They also change the number of cyclopentyl moieties or the position of branched methyl groups to adapt to the soil pH change (Ding et al., 2015; Weijers et al., 2007).

Archaeal membrane lipids. Different from the structure of brGDGTs produced by some specific bacteria, archaea synthesize monolayer membrane lipids, which contain two C_{40} isoprenoid chain carbon skeletons that are linked by an ether bound to glycerol and the head groups (Kate, 1993; Koga and Morii, 2005). Thus, they are called isoprenoid GDGTs (isoGDGTs). They are known to be synthesized by some methanogenic Euryarchaeota and Thaumarchaeota (Pitcher et al., 2010; Schouten et al., 2012). Their carbon chains contain 0–3 cyclopentane moieties and crenarchaeol which, in addition to 4 cyclopentane moieties and an extra cyclohexane ring (Schouten et al.,

2000). In addition, they also biosynthesize a trace amount of a regioisomer of crenarchaeol. According to the cultured study, hyperthermophilic archaea adjusted the number of cyclopentane moieties in their membrane lipids to adapt to the growth temperature (Gliozzi et al., 1983; Uda et al., 2001).

Over the past decade, their structural diversity, distribution, sources and the effect of environmental parameters are well studied in a wide range of nature, such as soils/peats, hot spring, lacustrine and marine suspended particulate matter (SPM) and sediments (Bechtel et al., 2010; Blaga et al., 2009; Kim et al., 2008; Loomis et al., 2011; Pitcher et al., 2011; Weber et al., 2015). The neutral fraction of archaea GDGT lipids which contain only core glycerol skeleton, is thought to be the fossilized remnants of the cellular material while their intact polar lipid (IPL) fraction with headgroup are thought to derived from living cells (Schouten et al., 2010). Upon cell lysis, the polar headgroups of most intact polar lipids are quickly degraded (Harvey et al., 1986; White et al., 1979), thus the presence of IPLs in an environment can be used as an indicator of active, living organisms (Lipp and Hinrichs, 2009; Lipp et al., 2008; Sturt et al., 2004). The IPLs in marine subsurface sediment contain mostly glycol- instead of phospho-headgroups (Lipp et al., 2008). Phospho-IPLs have been shown to degrade rapidly upon death of the source organism (Harvey et al., 1986; White et al., 1979), whereas the degradation kinetics of the more stable glyco-IPLs remains to be constrained. Recently, a laboratory experiment of IPL degradation test indicated that the bacterial glycerol ester lipids were faster degraded than archaeal ethers, but there's no relationship between the bonding type of the head group and the degradation rate (Logemann et al., 2011). The differences in chemical stability of IPLs play only a minor role during the degradation of IPLs, microbiological enzymatic processes are the driving force in IPL degradation.

1.3.2 Lipidomics based biomarkers

Microbial membrane lipids and their related fatty acids are useful biomarkers: 1) as they are major components of microbial living cells, their lipid profiles could provide insights into microbial communities since the certain lipids and their fatty acids differ among individual species; 2) their composition and structure respond well to the ambient environmental changes thus they could be used as tools for paleoclimate parameters reconstruction (Schouten et al., 2013; Zelles, 1997). For example, PLFAs 16:1$w8$ and 18:1$w8$ can be used as markers for methanotrophic bacteria (Sundh et al., 2000). The ratio of phospholipid PC to aminolipid DGTS could be used to evaluate the phosphorus condition (Geiger et al., 2010a). Crenarchaeol may be used a biomarker lipid for ammonium-oxidizing Thaumarchaeota (Pitcher et al., 2010). As mentioned above, intact polar lipids are potentially tracers for living bacteria and archaea due to a much shorter turn over timescale than their core lipid (Lipp et al., 2008; Schouten et al., 2012). To some extent, archaea intact polar lipids could also be used as an indicator of marine deep subsurface sedimentary biomass since archaea dominant in this region (Lipp et al., 2008).

The TEX_{86} (TetraEther indeX of tetraethers consisting of 86 carbon atoms) proxy based on archaea membrane lipids isoGDGTs, shows an empirical correlation with past sea surface temperature (Schouten et al., 2007). The ratio of dominant brGDGTs and isoGDGTs which called BIT (the branched isoprenoid tetraether) is frequently used as a proxy of terrestrial organic matter (OM) input into lakes or sea (Hopmans et al., 2004). In addition, the methylation index of branched tetraethers (MBT) and cyclization ratio of branched tetraethers (CBT) based on bacterial membrane lipids brGDGTs can be used to infer mean annual air temperature (MAT) and soil pH (Peterse et al., 2012; Weijers et al., 2007). By analyzing global soils, Weijers et al. (2007) found that MBT was controlled by mean annual air temperature (MAT) and to less extent by soil pH, whereas CBT only related to soil pH. Such relationship was calibrated and improved

by the subsequent study of Peterse et al. (2012) who proposed a simplified format of MBT (called MBT') based on seven quantifiable brGDGTs. However, comparing with other paleoclimate proxies like UK37 and $\delta^{18}O$, MBT-CBT index still exists very large scatter (> 5 °C, Schouten et al., 2013). The main factor to cause such a large calibration error is the co-elution of isomers. Thus, the improvement of the technics is needed. Ding et al. (2015) suggested the isomerization of 5-methyl to 6-methyl brGDGTs (named IBT) maybe another strategy for brGDGTs-producing bacteria to adapt their membrane fluidity to the ambient pH change.

To summarize, microbial membrane lipids are becoming more and more important for biomarkers in the field of biogeochemistry and paleoclimate (Peterse et al., 2009; Pitcher et al., 2009; Powers et al., 2010; Sinninghe Damsté et al., 2008).

1.4 Scope and Outline

This PhD thesis is connected to two projects. One is the Collaborative Research Centre AquaDiva (CRC 1076 AquaDiva) of the Friedrich Schiller Universität Jena. The other one is the Jena Experiment. Both of them are funded by the Deutsche the surface signal can be traced in the critical zone while the main objective of the Jena Experiment is to explore mechanisms underlying biodiversity and ecosystem functioning. Within the framework of both projects, this thesis focused on the information of the distribution and function of microbial communities in the research side by analyzing membrane lipids. The major goals are to improve the lipid-based markers for paleoclimate reconstruction and investigate the biological source and fate of microbial lipids in the critical zone as well as in surface soils.

This thesis is designed into two Parts: In Part I, I investigated the bacterial and archaeal GDGTs in different environments (soils, lake sediments and groundwater), evaluated their microbial community and environmental factors. Part II explores the full membrane lipidomics in the surface soils and their sources, potential use as

biomarkers. Part I has two first-authored manuscripts (both published) and Part II contains one first-authored manuscript (submitted). The main findings of this thesis are summarized behind.

Part I: GDGTs in lake sediments, groundwater and potential recharge surface soils

In Chapter 2, I analyzed brGDGTs using 102 surface lake sediments from thirty-seven Chinese lakes. By using improved chromatographic method, I found some novel isomers eluted after the previous known ones in Chinese lakes, which means previous identified 6-methyl brGDGTs were acctually many isomers co-eluted together. The complexity of the GDGT distribution and the relative abundance of the isomers resulted in large analytical uncertainties. Therefore, a seperation and identification of new isomers is needed to understand environmental influences on the new isomers as well as the previous known brGDGTs in the environment. I tentatively showed the structures of new isomers using an optimized HPLC gradient. Furthermore, I evaluated the environmental factors on these new brGDGT isomers.

In Chapter 3, I investigated the source and distribution of both core and intact polar bacterial and archaea GDGTs in groundwater. By comparing to the GDGTs in the soils of the potential recharge area, I showed that both bacterial and archaea GDGTs are mainly produced in situ in groundwater. Additionally, I showed that brGDGT-producing bacteria might be more active and thus have higher regeneration rates than the isoGDGT-producing archaea in both soils and groundwater. This is the first time for GDGTs study in groundwater and I proposed their potential use for evaluating soil inputs into terrestrial subsurface.

Part II: Full lipid profile and their affect environmental parameters in soils

In Chapter 4, I analyzed the full membrane lipid profile in the soils of a grassland. By comparing lipids from plant roots, fungi, Collembola and amoebae, I aim to first determine the source of these lipids in soils. In total, 380 membrane intact polar lipids from 16 major classes were identified in soils, much more than those found in plant

roots, fungi and Collembola. Then I evaluated the affect environmental parameters on the composition of these membrane lipids in soils. I showed plant diversity and block effect are the major factors for the change of lipid profile. This biomarker shows potential possibility to better understand soil microbial community by keeping their entire membrane lipid structures. In addition, the impact of plant diversity on the lipid spectrum in soils will reveal more detailed insights in the carbon cycling and carbon storage processes.

Part I. GDGTs in lake sediments, groundwater and potential recharge surface soils

2. Identification of novel 7-methyl and cyclopentanyl branched glycerol dialkyl glycerol tetraethers in lake sediments

Su Ding, Valérie F. Schwab, Nico Ueberschaar, Vanessa-Nina Roth, Markus Lange, Yunping Xu, Gerd Gleixner, Georg Pohnert

Published in *Organic Geochemistry* (2016), 102, 52-58

Manuscript I

Statement on individual contributions of doctoral candidate in a cumulative doctoral thesis

Publication (complete citation): **Ding, S.[1]**, Schwab, V.F.[2], Ueberschaar, N.[3], Roth, V.-N.[4], Lange, M.[5], Xu, Y.[6], Gleixner, G.[7], Pohnert, G.[8], 2016. Identification of novel 7-methyl and cyclopentanyl branched glycerol dialkyl glycerol tetraethers in lake sediments. *Organic Geochemistry* 102, 52-58								
Involved in	Author number							
	1	2	3	4	5	6	7	8
Conception of research approach	X	X				X	X	
Planning of research activities	X	X				X	X	X
Data collection	X		X					
Data analysis and interpretation	X	X		X	X	X	X	X
Writing a manuscript	X							X
Suggested publication equivalence value to be	1.0							

The subsequent manuscript has not been used in any other cumulative PhD thesis. Reprint permissions for this publication were obtained from the publisher and the coauthors.

Organic Geochemistry 102 (2016) 52–58

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Identification of novel 7-methyl and cyclopentanyl branched glycerol dialkyl glycerol tetraethers in lake sediments

Su Ding [a], Valérie F. Schwab [b], Nico Ueberschaar [b], Vanessa-Nina Roth [a], Markus Lange [a], Yunping Xu [c,d,*], Gerd Gleixner [a], Georg Pohnert [b,e,*]

[a] Max Planck Institute for Biogeochemistry, Hans-Knöll-Str. 10, 07745 Jena, Germany
[b] Institute for Inorganic and Analytical Chemistry, Bioorganic Analytics, Department of Chemistry and Earth Sciences, Friedrich-Schiller-Universität, Lessingstr. 8, 07743 Jena, Germany
[c] MOE Key Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, 100871 Beijing, China
[d] Shanghai Engineering Research Center of Hadal Science and Technology, College of Marine Sciences, Shanghai Ocean University, 201306 Shanghai, China
[e] Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany

ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form 22 September 2016
Accepted 24 September 2016
Available online 1 October 2016

Keywords:
7-Methyl brGDGTs
Biomarker
Lake sediments
Membrane lipids
Liquid chromatography–mass spectrometry

ABSTRACT

Branched glycerol dialkyl glycerol tetraethers (brGDGTs) are bacterial membrane lipids that are widely used as valuable paleoenvironmental proxies. The recently discovered 6-methyl brGDGTs improved the accuracy of the proxies for temperature "methylation branched tetraethers (MBT)" and soil pH "cyclization branched tetraethers (CBT)". However, the calibration uncertainties are still substantial for brGDGT-derived proxies (e.g., 5 °C for MBT'_{5ME}). Here we report a series of novel 7-methyl brGDGT isomers that co-eluted with the known 5- and 6-methyl brGDGTs in commonly applied normal phase high performance liquid chromatography (HPLC). Using an optimized HPLC gradient the novel 7-methyl brGDGTs could be structurally characterized and quantified. Their mean relative abundance was in the range of 6% of the total brGDGTs in Chinese and Cameroon lake sediments. The 7-methyl brGDGT IIa_7 correlates with sediment pH (R^2 = 0.44, root-mean-square error = 0.26 pH unit), a result that motivates the re-analysis of brGDGTs in soils and sediments to further reassess brGDGT-based proxies and to determine the source of 7-methyl brGDGTs. In addition to the 7-methyl brGDGTs, we identified two novel pentamethylated brGDGTs based on the mass spectra of its ether-cleaved hydrocarbon products.

1. Introduction

Branched glycerol dialkyl glycerol tetraethers (brGDGTs), which contain 4–6 methyl groups and up to two cyclopentane rings, are ubiquitously found in soils, peats, lakes and marginal seas (e.g., Weijers et al., 2007; Sinninghe Damsté et al., 2011; Hu et al., 2012; Günther et al., 2014; De Jonge et al., 2015b). Although these branched membrane lipids are detected in some *Acidobacteria* (Weijers et al., 2009; Sinninghe Damsté et al., 2011), their sources in the environment remain unknown (Anderson et al., 2014; Buckles et al., 2014; Naeher et al., 2014; Zell et al., 2014; De Jonge et al., 2016). By using liquid chromatography–mass spectrometry (HPLC–MS) and high-field nuclear magnetic resonance spectroscopy, Sinninghe Damsté et al. (2000) first discovered a group of GDGTs with branched carbon skeletons in peats, lakes, and marine sediments. In the same year, Schouten et al. (2000) reported another type of brGDGTs with one cyclopentane ring in addition to methyl group branches. Later, Weijers et al. (2006) tentatively identified additional brGDGT isomers with 0–2 cyclopentane rings, increasing the number of groups of brGDGT isomers up to 9. A survey of globally distributed soils revealed that the relative amount of methyl-branched tetraethers (MBT) correlates with mean annual air temperature (MAT) while cyclized branched tetraethers (CBT) correlate with soil pH (Weijers et al., 2007). However, the environmental temperature reconstruction using these proxies results in considerable scatter (ca. 5 °C for MAT) (Schouten et al., 2013). Progress in the field was made by improving the HPLC separation of brGDGTs. After Liu et al. (2012) reported a first 6-methyl brGDGT in marine sediments, De Jonge et al. (2013) unambiguously identified several novel 6-methyl brGDGTs from a Siberian peat core. These novel compounds co-eluted with previously known 5-methyl brGDGTs using conventional separation protocols. Becker et al. (2013) found novel brGDGT-III isomers (*m/z* 1050) in Aarhus

* Corresponding authors at: MOE Key Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, 100871 Beijing, China (Y. Xu). Institute for Inorganic and Analytical Chemistry, Bioorganic Analytics, Department of Chemistry and Earth Sciences, Friedrich-Schiller-Universität, Lessingstr. 8, 07743 Jena, Germany (G. Pohnert).
E-mail addresses: ypxu@shou.edu.cn (Y. Xu), Georg.Pohnert@uni-jena.de (G. Pohnert).

http://dx.doi.org/10.1016/j.orggeochem.2016.09.009
0146-6380/

Bay sediments by utilizing ultrahigh performance liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry (UHPLC–APCI-MS^2), but the exact positions of the methyl groups were not clarified. Weber et al. (2015) identified a novel hexamethylated brGDGT-$IIIa_{5/6}$ in a Swiss mountain lake. The $\delta^{13}C$ values of the alkyl chains indicated that it was likely produced in-situ in the lake's water column or sediment. Samples from a diversity of environments such as loess-paleosol sequences (e.g., Zech et al., 2012), soils (e.g., Yang et al., 2015) and marine sediments (e.g., Sinninghe Damsté, 2016) could be successfully separated and investigated. Different types of LC columns used include, for example, two BEH amide columns (Becker et al., 2013) or two UHPLC silica columns in tandem (Yang et al., 2015). Hopmans et al. (2016) compared different HPLC protocols and came up with an improved chromatography using two UHPLC silica columns in series. Remarkably, GDGT-based proxies differed for MBT_{5ME} determination depending on the protocol used for separation (ca. 3 °C deviation when used for MAT reconstruction). With improvements in chromatography, the number of brGDGTs detected increased from 9 to 15, and several modified proxies containing 6-methyl brGDGTs were proposed for reconstruction of soil pH and MAT (Ding et al., 2015; Xiao et al., 2015; Wang et al., 2016).

Numerous studies utilize the methylation pattern of brGDGTs for environmental correlations. Dang et al. (2016), for example, found a moisture control on 6-methyl brGDGT distributions in semi-arid and arid soils. Results of the analyses of sources and distributions of 6-methyl brGDGTs in coastal marine and lake sediments suggest that brGDGTs are universally produced in the water column (De Jonge et al., 2015a, 2015b, 2016; Sinninghe Damsté, 2016).

These results suggest that the adjustment of membrane lipids to temperature and pH are manifold and that still further methylation, cyclization, and isomerization patterns may exist. Their discovery may improve the quality of our calibrations for environmental parameters. Here we report a series of new 7-methyl isomers that eluted after 5- and 6-methyl brGDGTs which were characterized by a modified HPLC separation combined with fraction collection and identification of ether cleavage hydrocarbon products in extracts from Chinese lake sediments. Furthermore, pentamethylated brGDGTs with one cyclopentane ring (IIb, *m/z* 1034) were found to comprise two different structures in a single HPLC peak. A lake sediment from Baleng in Cameroon was used to test if 7-methyl brGDGTs can be found in other lakes.

2. Materials and methods

2.1. Sampling

In July 2014, a total of 102 surface lake sediments (0–5 cm) were collected from 37 Chinese lakes with a grab sampler. The Chinese lakes span a large longitude and latitude range (86° 51′11″–132°31′33″E, 24°54′16″–49°18′17″N; for the exact sampling location see Supplementary Table 1), displaying a wide range of MAT (−0.7 to 17.5 °C) and mean annual precipitation (MAP, 77–1323 mm). The MAT and MAP values (1960–1990) were obtained from the WorldClim data set (Hijmans et al., 2005). The water pH of these lakes varies from 6.9 (Poyang Lake) to 10.2 (Tai Lake) with an average value of 8.3 suggesting that most lakes are alkaline. The lake sediment of Baleng was collected in November 2011 according to Garcin et al. (2012). After collection, all samples were stored at −20 °C until analysis.

2.2. brGDGTs purification and collection

Surface sediments (each ca. 6 g) were freeze-dried, extracted and concentrated according to Ding et al. (2015). All the samples were dissolved in 300 μL of a mixture of 84% *n*-hexane (A) and 16% ethyl acetate (B). A 10 μL portion of each sample was injected on an Agilent 1200 series HPLC system coupled to a Thermo Scientific Orbitrap Velos Pro ion trap mass spectrometer for the determination of individual brGDGT fractional abundance. Two Hypersil GOLD silica columns (150 mm × 2.1 mm; 1.9 μm; Thermo Fisher Scientific, USA) in series were used by means of a modified method based on the protocol of Yang et al. (2015). The HPLC was operated at a constant flow rate of 0.2 mL/min. The initial solvent composition was held at 84% A and 16% B for 65 min, followed by an increase to 100% B within 20 min before re-equilibration at 84% A and 16% B for 30 min. Atmospheric pressure chemical ionization (APCI) settings were according to De Jonge et al. (2013), spectra were recorded in SIM mode in the mass range of *m/z* 1017–1051 (2 μscans; 100 ms max injection time).

After the quantification of brGDGTs from all the lake sediments, 66% of each sample were taken, combined and concentrated to dryness under a stream of nitrogen. Two HPLC purification steps were conducted for further fraction collection. First, the combined sample was re-dissolved in ca. 5 mL of the HPLC solvent mixture consisting of 84% A and 16% B. Repeated injections of 100 μL were performed on HPLC–MS with one Hypersil GOLD silica column. The HPLC was operated isocratically using 84% *n*-hexane (A) and 16% ethyl acetate (B) at a flow rate of 0.5 mL/min. The HPLC outlet was equipped with a 1:10 splitter (mass spectrometer: manual fraction collection) and connected to the APCI-MS. The brGDGT fractions were combined and concentrated under a stream of nitrogen.

Secondly, the pre-purified brGDGTs were re-dissolved in 200 μL of a mixture of 84% A and 16% B for further purification. Aliquots of 10 μL of this mixture were injected on two Hypersil GOLD silica columns in series. The HPLC–MS conditions were the same as in the quantification method described above. Two fractions were collected (fraction A from 45.2 to 47.0 min and fraction B from 49.7 to 53.0 min; Fig. 1A and B). For further quantitative HPLC–MS analysis, a 10% aliquot of each fraction was dried under a stream of nitrogen and re-dissolved in 250 μL of 84% A and 16% B. 50 μL of an 11.9 ppm (ng/μL) solution of the C_{46} GDGT was added as an internal standard (Huguet et al., 2006). We did not achieve baseline separation of the novel brGDGT isomers that co-eluted with other brGDGTs of different mass. Nonetheless, these mixtures were sufficiently pure for detailed analysis of hydrocarbon moieties.

2.3. Ether cleavage of brGDGTs and analysis

The remaining 90% of two specific fractions (see Section 2.2; Fig. 1A and B) were submitted to ether cleavage using 57 wt% HI in H_2O followed by reduction to hydrocarbons using H_2/PtO_2 according to published procedures (Kaneko et al., 2011; De Jonge et al., 2013). The products were separated on a ThermoTrace 1310 gas chromatograph (Bremen, Germany) coupled with a Thermo TSQ 8000 electron impact (EI) triple quadrupole mass (GC–MS). The PTV injector was operated in splitless mode at an initial temperature of 70 °C. With injection, the injector was heated to 300 °C at a programmed rate of 720 °C/min and held at this temperature for 2.5 min. Separation was performed on a Thermo TG-5SilMS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The GC oven temperature was held at 100 °C for 1 min and subsequently raised to 320 °C at a rate of 5 °C/min. This temperature was held for 3 min before cooling and re-equilibration. Data acquisition started at 10 min, monitoring the mass range between 50 and 650 Da in EI+ (70 eV) mode. The MS transfer line and the ion source temperature were set to 300 °C.

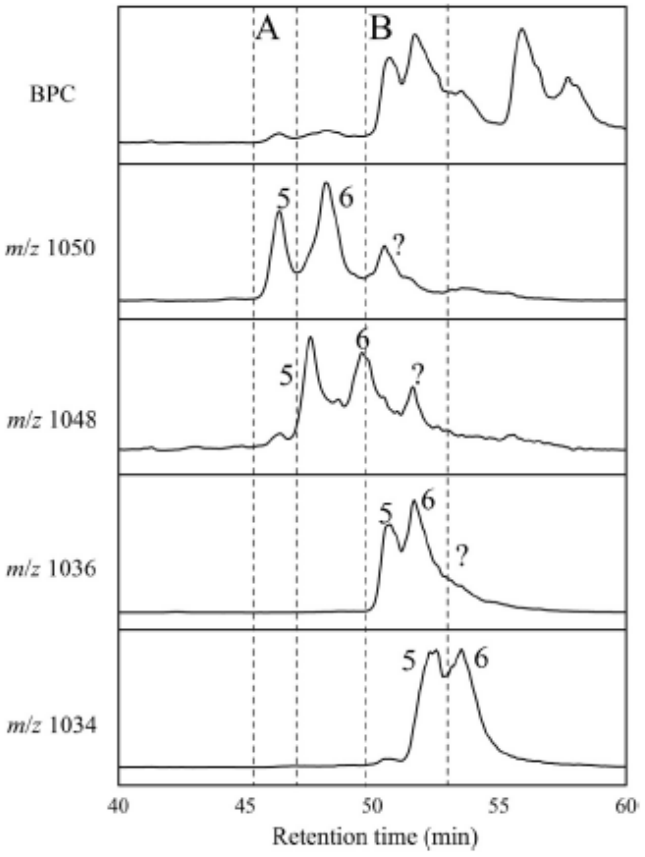


Fig. 1. HPLC–APCI-MS chromatograms of brGDGTs in Chinese lake sediments including base peak chromatogram (BPC) *m/z* 1050, 1048, 1036 and 1034 extracted ion chromatograms. Numbers represent the 5- and 6-methylated isomers of brGDGTs while the question mark represents the newly identified isomers.

3. Results and discussion

3.1. Identification of 7-methyl brGDGTs

The modified HPLC method allows the separation of new brGDGT isomers from the known 5- and 6-methyl brGDGTs with the same mass (Fig. 1). To identify the structure of the unknown compounds, the two fractions of brGDGTs from Chinese lake sediments were subjected to ether cleavage and the hydrocarbons generated were analyzed using GC–MS. To initially monitor the success of fractionation and ether cleavage we focused on fraction A (Fig. 1) that did not contain any novel brGDGTs. In this fraction we detected a mixture of the well-described lipids 5-methyl brGDGT (*m/z* 1050, $IIIa_5$), 5/6-methyl brGDGT (*m/z* 1050, $IIIa_{5/6}$) and 6-methyl brGDGT (*m/z* 1050, $IIIa_6$) (Supplementary Fig. S1). Thus this fraction was ideally suited for method verification. After cleavage of the ether bonds, five hydrocarbons arising from the brGDGTs could be detected (Fig. 2a–e). The most abundant two isomers were 6,13,16-trimethyloctacosane (b) and 5,13,16-trimethyloctacosane (c), and the other three minor compounds were 13,16-dimethyloctacosane (a), 6,13,16,24-tetramethyloctacosane (d) and 5,13,16,24-tetramethyloctacosane (e) (Fig. 2; fraction A). All hydrocarbons were identified according to their specific fragmentation patterns in EI mass spectra and according to their retention time (Fig. 2a–e). These data were in good agreement with Sinninghe Damsté et al. (2000), Liu et al. (2012) and De Jonge et al. (2013). However, 5,13,16,23-tetramethyloctacosane was not reported in previous studies, presumably due to its low concentration (Weber et al., 2015). The composition of methylated hydrocarbons released during ether cleavage thus reflects directly the composition of their parent brGDGTs, supporting the applicability of this approach for the identification of unknown tetraethers.

Fraction B (Fig. 1 and Table 1) mainly contained unknown brGDGT isomers (*m/z* 1050, 1048 and 1036) as well as known 5-methyl brGDGTs (*m/z* 1036, IIa_5; *m/z* 1034, IIb_5) and 6-methyl brGDGTs (*m/z* 1036, IIa_6; *m/z* 1034, IIb_6). Hydrocarbons derived from the ether cleavage were separated using GC–MS (Fig. 2; fraction B). The earlier eluting compounds were assigned as 13,16-dimethyloctacosane (a), 6,13,16-trimethyloctacosane (b) and 5,13,16-trimethyloctacosane (c), which are also present in fraction A. Mass spectra of hydrocarbons f–h, however, indicated the presence of a cyclopentane ring. While f is mono-methylated, both g and h contained an additional methyl group at position 5 and 6 of the alkyl chain, respectively. Compound f was hitherto only described as its methylthioether derivative (Schouten et al., 2000), but is here for the first time supported by a mass spectrum of the non-functionalized hydrocarbon (Fig. 2). In addition, we observed a new compound i which eluted before hydrocarbon b. Although the abundance of i was relatively small, its mass spectrum clearly showed differences from that of b and c, i.e., increased intensity of fragments at *m/z* 351 and 323 in i, but higher intensity of fragments at *m/z* 365 and 379 in b and c, respectively (Table 2). Such fragmentation pattern suggests that hydrocarbon i is methylated at C-7 of its alkyl chain rather than at C-5 or C-6. The comparison of the extracted ion chromatogram (*m/z* 351.3, red solid line in Fig. 2; fractions A and B) clearly confirms that the peak shoulder at retention time 34.2 min in Fig. 2 fraction B corresponds to a defined peak, which further proves the existence of a defined hydrocarbon with 7-methyl substitution. In contrast, the signal of the same ion trace at similar retention time in Fig. 2 fraction A does not have a defined shoulder.

The error (7 units) between the measured (2895) and calculated (2902) Kováts index of 7,13,16-trimethyloctacosane was comparable to that of 5,13,16-trimethyloctacosane (6 units) and lower than the error of 6,13,16-trimethyloctacosane (12 units) (Kissin et al., 1986), which further confirmed the structure of the newly identified carbon skeleton i (Table 1). This hydrocarbon thus clearly supports the presence of 7-methyl brGDGTs in lake sediments (Fig. 3). Furthermore, the elution times of 7-methyl brGDGTs in HPLC–MS analysis were in accordance with their structural properties (i.e., the elution order of IIIa is listed as $IIIa_5$, $IIIa_{5/6}$, $IIIa_6$ and finally $IIIa_7$ as shown in Supplementary Fig. S2; see also Weber et al., 2015).

LC–MS and GC–MS data provide semi-quantitative information about the respective brGDGTs and their hydrocarbon cleavage products. Assuming similar response factors for the different brGDGTs in LC–MS as well as for the hydrocarbons in GC–MS analyses, our data allows an evaluation of the proportions of the respective lipids. The relative abundance of brGDGTs in fraction B is given in Table 1 and the relative abundance of the hydrocarbons generated by ether cleavage of fraction B in Table 3. Formally, three hydrocarbons could be generated by cleavage of two isomeric forms of $IIIa_7$ (a, i and 7,13,16,22-tetramethyloctacosane; Fig. 3). However, the latter hydrocarbon was not detected which may be due to the absence or low concentration of the corresponding isomer ($IIIa^{?}_{7}$). Therefore, brGDGT-$IIIa_7$ generated two equivalents of hydrocarbon i. Hydrocarbon i accounts for 4.5% of total hydrocarbons released from fraction B, supporting the presence of a substantial proportion of the novel 7-methyl branched GDGTs. The similar retention time interval between structures $IIIa_5$, $IIIa_6$, and the novel compound during LC–MS analysis further supports the presence of two methyl branches in $IIIa_7$ as similarly reported for $IIIa_5$ and $IIIa_6$. Since we have not detected any other peaks eluting in between (as observed for the previously identified mixed compound $IIIa_{5/6}$) we exclude branching at other positions.

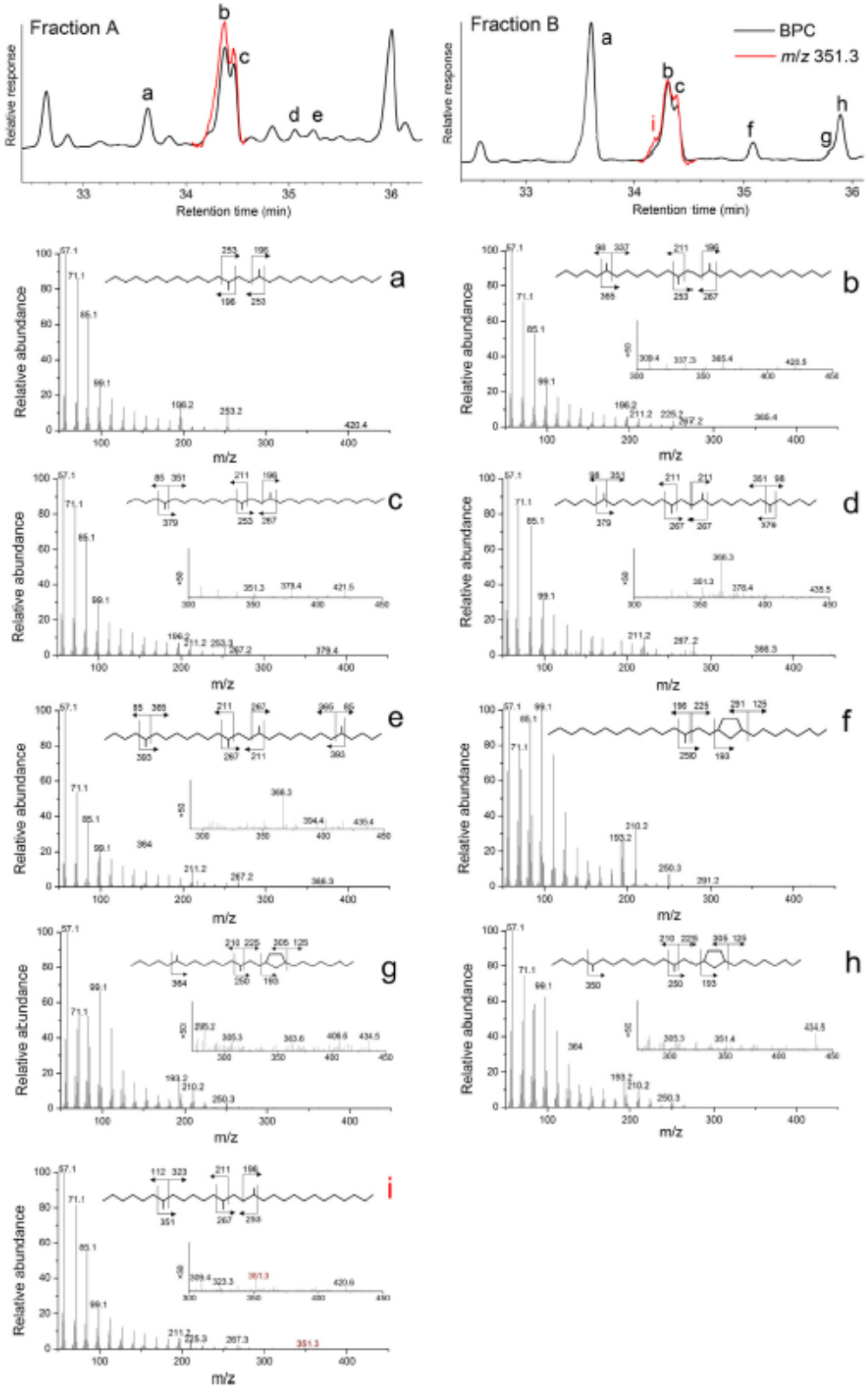


Fig. 2. GC–MS chromatograms and corresponding mass spectra of hydrocarbons (a–i) generated by ether cleavage of the two brGDGT fractions A and B. Both BPC and extracted ion chromatogram (*m/z* 351.3, red solid line) are shown in the chromatogram of fraction A and of fraction B. Mass spectra and hydrocarbon structures (a–i) are shown, with preferred fragmentation indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The ratios of carbon skeletons i/(a + f) with respect to brGDGTs before ether cleavage (based on HPLC–MS peak integration) and their ether-cleavage products hydrocarbons i, a and f (based on GC–MS peak integration) were calculated to further test whether all three new isomers (Fig. 1; *m/z* 1050, 1048, 1036) were 7-methyl brGDGTs:

Table 1
Relative abundance of brGDGTs in fraction B (see Fig. 1) evaluated by integration of the respective ion traces during LC–MS analysis. Predicted hydrocarbons arising from ether cleavage are indicated in the last column.

brGDGT type	Relative abundance (%)	*m/z*	Contribute to fragments
$IIIa_7$	2.6	1050	i
$IIIb_6$	0.3	1048	b, g
$IIIb_7$	0.8	1048	i
IIa_5	17.7	1036	c, a
IIa_6	49.3	1036	b, a
IIa_7	3.8	1036	i, a
IIb_5	20.3	1034	c, f (h, a)
IIb_6	5.2	1034	b, f (g, a)

Table 3
Relative abundance of hydrocarbons generated by ether cleavage of fraction B (see Fig. 1).

Carbon skeleton	Relative abundance (%)
a	43.3
i	4.5
b	22.2
c	12.2
f	4.4
g	1.8
h	11.6

$$\text{Before ether cleavage: } i/(a+f) = (IIIa_7 \times 2 + IIIb_7 + IIa_7)/(IIa_5 + IIa_6 + IIa_7 + IIb_5 + IIb_6) = 0.102$$

$$\text{After ether cleavage: } i/(a+f) = 0.102$$

The similar ratios observed before and after the ether cleavage are additional support for the 7-methyl brGDGTs structure of the new isomers (Fig. 3).

3.2. Identification of two pentamethylated brGDGTs' structural isomers

Besides novel 7-methyl compounds, we tentatively identified novel structural isomers of the cyclopentane-containing pentamethylated brGDGT isomers IIb (*m/z* 1034, IIb_5 and IIb_6). IIb_5 and IIb_6 would liberate hydrocarbons b/c and f. Previous studies treated IIb_5 as a single compound contributing to hydrocarbons c and f (e.g., Weijers et al., 2007). However, we found hydrocarbons g and h in fraction B that could only be related to IIb_5 and IIb_6. Therefore we assume that IIb_5 and IIb_6 both consist of two structural isomers (Fig. 4); IIb_5 contains brGDGTs with hydrocarbons c and f or g and a, while IIb_6 is composed of hydrocarbons b and f or h and a. Evidence that the sum of the relative abundance of hydrocarbons a and f (43.9%, another 3.8% contributed to one chain of IIa_7) is similar to the sum of b, c, g and h (47.8%: these four hydrocarbons have to be located in the second chain, besides a or f, of the brGDGTs) confirms our hypothesis.

3.3. Method evaluation

In order to evaluate our modified method, we analyzed one of the lake samples from Bosten Lake (BSTH-D4) in comparison to the HPLC method reported by Hopmans et al. (2016). Retention times using our method were longer compared to the ones reported by Hopmans et al. (2016), while the sensitivity of both methods was comparable. Both methods allow the separation of 7-methyl brGDGTs, sufficient for quantification (Supplementary Fig. S2). However, only using our method, $IIIa_{5/6}$ could be seen as a shoulder peak on $IIIa_6$. This isomer co-eluted with $IIIa_6$ using chromatography from Hopmans et al. (2016). For exact quantification, peak deconvolution algorithms could be applied to the chromatograms obtained with our method.

3.4. Survey of Chinese and Cameroon lake sediments

The average fractional abundance of brGDGTs from 102 samples in 37 Chinese lakes is shown in Supplementary Fig. S3. The 7-methyl brGDGTs were detected in 92 out of 102 lake sediments and comprised on average 6.2% of the total amount of brGDGTs. For those sediments where 7-methyl brGDGTs were not detected, the total abundance of brGDGTs was very low and only a few brGDGTs were above the limit of detection (LOD). In some lake sediments, the relative abundance of 7-methyl brGDGTs can reach 11% of total brGDGTs (e.g., DC-D2 from Dai lake and HLH-D7 from Hunlun lake). The pH of these two samples (8.5 for DC-D2 and 8.2 for HLH-D7) was higher than those of the other investigated lake sediments. In the analysis of the correlation between sediment pH and individual brGDGTs in Chinese lake sediments, we found that IIa_7 shows the best correlation with sediment pH ($R^2 = 0.44$, root-mean-square error = 0.26 pH unit; Supplementary Fig. S4), suggesting that the carbon skeleton isomerization of branched tetraethers is an important strategy for brGDGTs-producing bacteria to adapt to ambient pH (Ding et al., 2015). According to our survey,

Table 2
Carbon skeletons generated from 5- to 7-methyl brGDGTs, their measured and calculated Kováts indexes and diagnostic fragment ions in the mass spectra.

brGDGT branched methyl position	Carbon skeleton	RI measured	RI calculated[a]	Diagnostic ions in mass spectrum
5	c	2905	2911	379, 351, 196, 211, 253, 267
6	b	2900	2912	365, 337, 196, 211, 253, 267
7	i	2895	2902	351, 323, 196, 211, 253, 267

[a] Using additivity principles for the calculation of relative retention factors (RRF) to obtain RI (calculated).

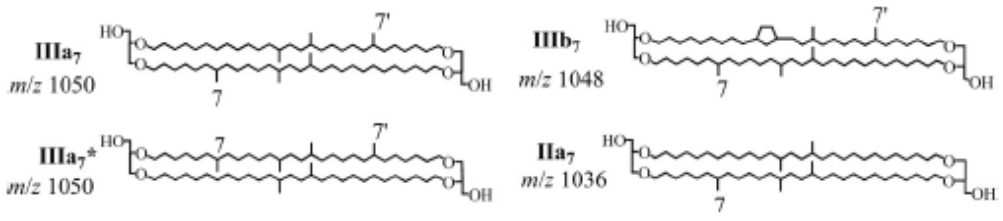


Fig. 3. Structures of newly identified 7-methyl brGDGTs (*m/z* 1050, 1048, 1036). All structures of novel compounds are tentatively identified because no pure standard compounds are available. $IIIa_7^*$ was not recognized due to absence or low concentration of 7,13,16,22-tetramethyloctacosane, $IIIb_7$ was identified by the similar ratio of i/(a + f) before (HPLC–MS) and after (GC–MS) ether cleavage (Section 3.2).

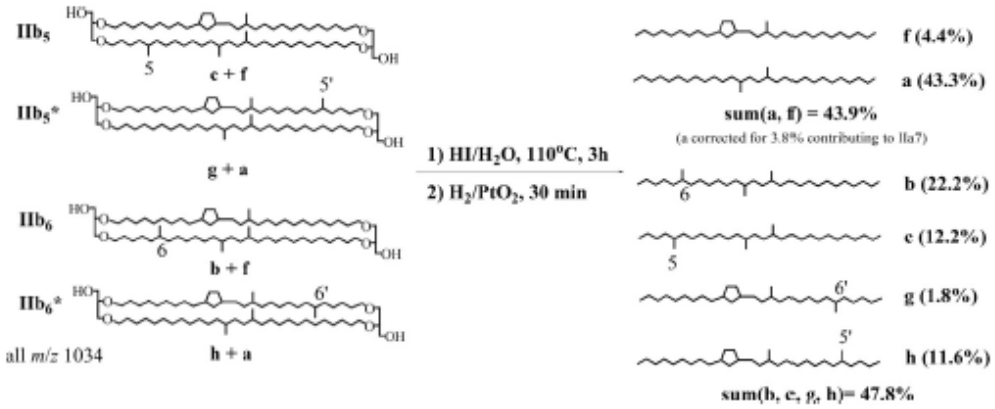


Fig. 4. Ether cleavage products of brGDGTs-IIb isomers. The conventional IIb_5 and IIb_6 are suggested to be composed of two structural isomers (IIbʇ and IIbʇ). Labels (a, b, c, f, g and h) correspond to the hydrocarbons in Fig. 2.

7-methyl brGDGTs appear ubiquitous in lakes, some like IIa_7 even with a fractional abundance above 5%. For comparison, a global soil dataset lists only 6 out of 15 brGDGTs with a fractional abundance above 5% ($IIIa_5$, $IIIa_6$, IIa_5, IIa_6, Ia and Ib) (De Jonge et al., 2014). The novel 7-methyl brGDGTs should thus be considered when brGDGT-proxies are further developed. Penta- and tetramethylated brGDGTs (IIa, IIb, Ia and Ib) showed relatively high fractional abundances (>10%) in our 102 Chinese lake sediment samples. 7-Me brGDGTs have not been encountered in a Siberian peat (De Jonge et al., 2013) or in a soil dataset (De Jonge et al., 2014), which can be either explained by their absence in soils or by the omission of the screening for these, then unknown, brGDGT isomers. Therefore, we suggest the careful reevaluation of brGDGTs in soils in order to clarify the exact source of 7-methyl brGDGTs in lakes such as those in our study.

To verify if 7-methyl brGDGTs are also found in other sediments than those of the lakes described above, we investigated a sediment sample from the Lake of Baleng in Cameroon (Fig. 5). The relative abundance of 7-methyl brGDGTs is comparable between the Lake of Baleng and the mean of the Chinese lakes (4.3% vs 6.2%), indicating that these compounds might be universally distributed and significant. This calls for 7-methyl brGDGTs to be considered when establishing new brGDGT proxies.

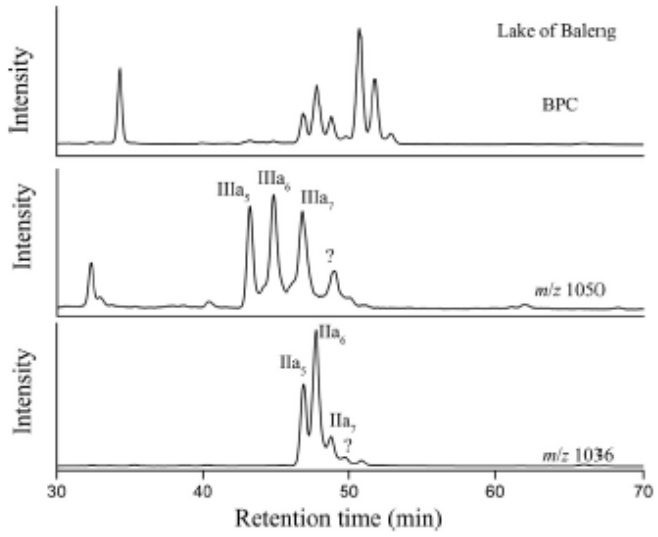


Fig. 5. HPLC–APCI-MS chromatograms of brGDGTs from the sediment of Lake of Baleng in Cameroon, including BPC, *m/z* 1050 and 1036 extracted ion chromatograms.

4. Conclusions

We identified a novel class of brGDGTs with methylation at C-7 that contribute 6.2% of the total brGDGTs in Chinese lake sediments ($IIIa_7$ 1.4%, $IIIb_7$ 0.3% and IIa_7 4.5% of total brGDGTs) and 4.3% in Lake of Baleng in Cameroon ($IIIa_7$ 1.4% and IIa_7 2.9% of total brGDGTs). IIa_7 shows the best correlation with sediment pH in Chinese lake sediments. This finding has important implications for the improvement of existing brGDGT-based pH proxies such as CBT and IBT. Follow-up studies should test for the presence of these novel compounds and examine whether 7-methyl brGDGTs could be attributed to specific environmental conditions or microorganisms. In addition, we found that the known brGDGTs (*m/z* 1034) with one cyclopentane moiety, IIb_5 and IIb_6, are actually composed of two structural isomers that can further complicate previously defined brGDGT-based proxies. The mechanism upon which bacteria produce different brGDGT membrane lipid isomers is still needed to be further studied based on improved HPLC methods that allow individual quantification of tetraether compounds.

Acknowledgments

We would like to thank Wei He, Wenxiu Liu, Bin Yang, Yinghui Wang and Weijie Xiao for help with the sampling of Chinese lake sediments. We thank Dr. Julius Lipp for the suggestion of the HPLC–MS method and APCI settings and Dr. Yannick Garcin for providing lake sediment of Cameroon sampled during the German Research Foundation (DFG) project GA-1629/1-1&2. We also thank two anonymous reviewers and the editor for helpful comments and suggestions. This work was financially supported by the DFG within the framework of the CRCs 1076 AquaDiva and 1127 ChemBioSys and National Science Foundation of China (41476062). The IMPRS-gBGC provided the funding for the PhD scholarship of Su Ding.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2016.09.009.

Associate Editor—**Marcus Elvert**

References

Anderson, V.J., Shanahan, T.M., Saylor, J.E., Horton, B.K., Mora, A.R., 2014. Sources of local and regional variability in the MBT′/CBT paleotemperature proxy: insights from a modern elevation transect across the Eastern Cordillera of Colombia. Organic Geochemistry 69, 42–51.

Becker, K.W., Lipp, J.S., Zhu, C., Liu, X.-L., Hinrichs, K.-U., 2013. An improved method for the analysis of archaeal and bacterial ether core lipids. Organic Geochemistry 61, 34–44.

Buckles, L.K., Weijers, J.W.H., Verschuren, D., Sinninghe Damsté, J.S., 2014. Sources of core and intact branched tetraether membrane lipids in the lacustrine environment: anatomy of Lake Challa and its catchment, equatorial East Africa. Geochimica et Cosmochimica Acta 140, 106–126.

Dang, X., Yang, H., Naafs, B.D.A., Pancost, R.D., Xie, S., 2016. Evidence of moisture control on the methylation of branched glycerol dialkyl glycerol tetraethers in semi-arid and arid soils. Geochimica et Cosmochimica Acta.

De Jonge, C., Hopmans, E.C., Stadnitskaia, A., Rijpstra, W.I.C., Hofland, R., Tegelaar, E., Sinninghe Damsté, J.S., 2013. Identification of novel penta- and hexamethylated branched glycerol dialkyl glycerol tetraethers in peat using HPLC–MS^2, GC–MS and GC–SMB-MS. Organic Geochemistry 54, 78–82.

De Jonge, C., Hopmans, E.C., Zell, C.I., Kim, J.-H., Schouten, S., Sinninghe Damsté, J.S., 2014. Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: implications for palaeoclimate reconstruction. Geochimica et Cosmochimica Acta 141, 97–112.

De Jonge, C., Stadnitskaia, A., Cherkashov, G., Sinninghe Damsté, J.S., 2016. Branched glycerol dialkyl glycerol tetraethers and crenarchaeol record post-glacial sea level rise and shift in source of terrigenous brGDGTs in the Kara Sea (Arctic Ocean). Organic Geochemistry 92, 42–54.

De Jonge, C., Stadnitskaia, A., Fedotov, A., Sinninghe Damsté, J.S., 2015a. Impact of riverine suspended particulate matter on the branched glycerol dialkyl glycerol tetraether composition of lakes: the outflow of the Selenga River in Lake Baikal (Russia). Organic Geochemistry 83–84, 241–252.

De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Streletskaya, I.D., Vasiliev, A.A., Sinninghe Damsté, J.S., 2015b. Drastic changes in the distribution of branched tetraether lipids in suspended matter and sediments from the Yenisei River and Kara Sea (Siberia): implications for the use of brGDGT-based proxies in coastal marine sediments. Geochimica et Cosmochimica Acta 165, 200–225.

Ding, S., Xu, Y., Wang, Y., He, Y., Hou, J., Chen, L., He, J.S., 2015. Distribution of branched glycerol dialkyl glycerol tetraethers in surface soils of the Qinghai-Tibetan Plateau: implications of brGDGTs-based proxies in cold and dry regions. Biogeosciences 12, 3141–3151.

Günther, F., Thiele, A., Gleixner, G., Xu, B., Yao, T., Schouten, S., 2014. Distribution of bacterial and archaeal ether lipids in soils and surface sediments of Tibetan lakes: implications for GDGT-based proxies in saline high mountain lakes. Organic Geochemistry 67, 19–30.

Garcin, Y., Schwab, V.F., Gleixner, G., Kahmen, A., Todou, G., Séné, O., Onana, J.-M., Achoundong, G., Sachse, D., 2012. Hydrogen isotope ratios of lacustrine sedimentary *n*-alkanes as proxies of tropical African hydrology: insights from a calibration transect across Cameroon. Geochimica et Cosmochimica Acta 79, 106–126.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25, 1965–1978.

Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2016. The effect of improved chromatography on GDGT-based palaeoproxies. Organic Geochemistry 93, 1–6.

Hu, J., Meyers, P.A., Chen, G., Peng, P.a., Yang, Q., 2012. Archaeal and bacterial glycerol dialkyl glycerol tetraethers in sediments from the Eastern Lau Spreading Center, South Pacific Ocean. Organic Geochemistry 43, 162–167.

Huguet, C., Hopmans, E.C., Febo-Ayala, W., Thompson, D.H., Sinninghe Damsté, J.S., Schouten, S., 2006. An improved method to determine the absolute abundance of glycerol dibiphytanyl glycerol tetraether lipids. Organic Geochemistry 37, 1036–1041.

Kaneko, M., Kitajima, F., Naraoka, H., 2011. Stable hydrogen isotope measurement of archaeal ether-bound hydrocarbons. Organic Geochemistry 42, 166–172.

Kissin, Y.V., Feulmer, G.P., Payne, W.B., 1986. Gas chromatographic analysis of polymethyl-substituted alkanes. Journal of Chromatographic Science 24, 164–169.

Liu, X.-L., Summons, R.E., Hinrichs, K.-U., 2012. Extending the known range of glycerol ether lipids in the environment: structural assignments based on tandem mass spectral fragmentation patterns. Rapid Communications in Mass Spectrometry 26, 2295–2302.

Naeher, S., Peterse, F., Smittenberg, R.H., Niemann, H., Zigah, P.K., Schubert, C.J., 2014. Sources of glycerol dialkyl glycerol tetraethers (GDGTs) in catchment soils, water column and sediments of Lake Rotsee (Switzerland) – implications for the application of GDGT-based proxies for lakes. Organic Geochemistry 66, 164–173.

Schouten, S., Hopmans, E.C., Pancost, R.D., Sinninghe Damsté, J.S., 2000. Widespread occurrence of structurally diverse tetraether membrane lipids: evidence for the ubiquitous presence of low-temperature relatives of hyperthermophiles. Proceedings of the National Academy of Sciences 97, 14421–14426.

Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. Organic Geochemistry 54, 19–61.

Sinninghe Damsté, J.S., 2016. Spatial heterogeneity of sources of branched tetraethers in shelf systems: the geochemistry of tetraethers in the Berau River delta (Kalimantan, Indonesia). Geochimica et Cosmochimica Acta 186, 13–31.

Sinninghe Damsté, J.S., Hopmans, E.C., Pancost, R.D., Schouten, S., Geenevasen, J.A., 2000. Newly discovered non-isoprenoid glycerol dialkyl glycerol tetraether lipids in sediments. Chemical Communications, 1683–1684.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Weijers, J.W.H., Foesel, B.U., Overmann, J., Dedysh, S.N., 2011. 13,16-Dimethyl octacosanedioic acid (iso-diabolic acid), a common membrane-spanning lipid of Acidobacteria subdivisions 1 and 3. Applied and Environment Microbiology 77, 4147–4154.

Wang, H., Liu, W., Lu, H., 2016. Appraisal of branched glycerol dialkyl glycerol tetraether-based indices for north China. Organic Geochemistry 98, 118–130.

Weber, Y., De Jonge, C., Rijpstra, W.I.C., Hopmans, E.C., Stadnitskaia, A., Schubert, C.J., Lehmann, M.F., Sinninghe Damsté, J.S., Niemann, H., 2015. Identification and carbon isotope composition of a novel branched GDGT isomer in lake sediments: evidence for lacustrine branched GDGT production. Geochimica et Cosmochimica Acta 154, 118–129.

Weijers, J.W.H., Schouten, S., Hopmans, E.C., Geenevasen, J.A.J., David, O.R.P., Coleman, J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. Environmental Microbiology 8, 648–657.

Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007. Environmental controls on bacterial tetraether membrane lipid distribution in soils. Geochimica et Cosmochimica Acta 71, 703–713.

Weijers, J.W.H., Panoto, E., van Bleijswijk, J., Schouten, S., Rijpstra, W.I.C., Balk, M., Stams, A.J., Damste, J.S.S., 2009. Constraints on the biological source(s) of the orphan branched tetraether membrane lipids. Geomicrobiology Journal 26, 402–414.

Xiao, W., Xu, Y., Ding, S., Wang, Y., Zhang, X., Yang, H., Wang, G., Hou, J., 2015. Global calibration of a novel, branched GDGT-based soil pH proxy. Organic Geochemistry 89–90, 56–60.

Yang, H., Lü, X., Ding, W., Lei, Y., Dang, X., Xie, S., 2015. The 6-methyl branched tetraethers significantly affect the performance of the methylation index (MBI′) in soils from an altitudinal transect at Mount Shennongjia. Organic Geochemistry 82, 42–53.

Zech, R., Gao, L., Tarozo, R., Huang, Y., 2012. Branched glycerol dialkyl glycerol tetraethers in Pleistocene loess-paleosol sequences: Three case studies. Organic Geochemistry 53, 38–44.

Zell, C., Kim, J.-H., Hollander, D., Lorenzoni, L., Baker, P., Silva, C.G., Nittrouer, C., Sinninghe Damsté, J.S., 2014. Sources and distributions of branched and isoprenoid tetraether lipids on the Amazon shelf and fan: implications for the use of GDGT-based proxies in marine sediments. Geochimica et Cosmochimica Acta 139, 293–312.

Supplementary material

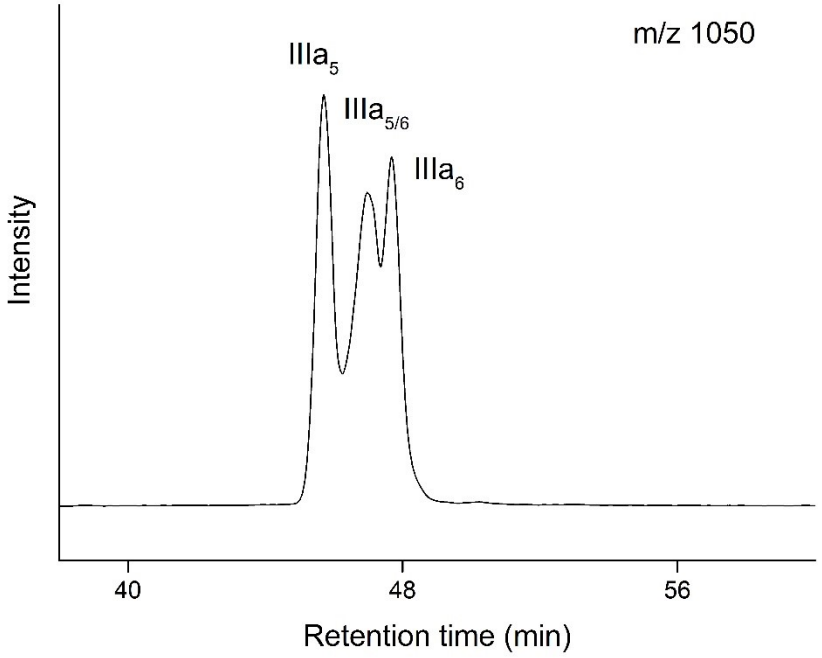


Fig. S1. Extracted ion chromatogram (*m*/*z* 1050) of fraction 1 (Fig. 1A) reran after fraction collection of Chinese lake sediments on HPLC/MS. 5-methyl brGDGT ($IIIa_5$), 5/6-methyl brGDGT ($IIIa_{5/6}$) and 6-methyl brGDGT ($IIIa_6$) were found in this fraction.

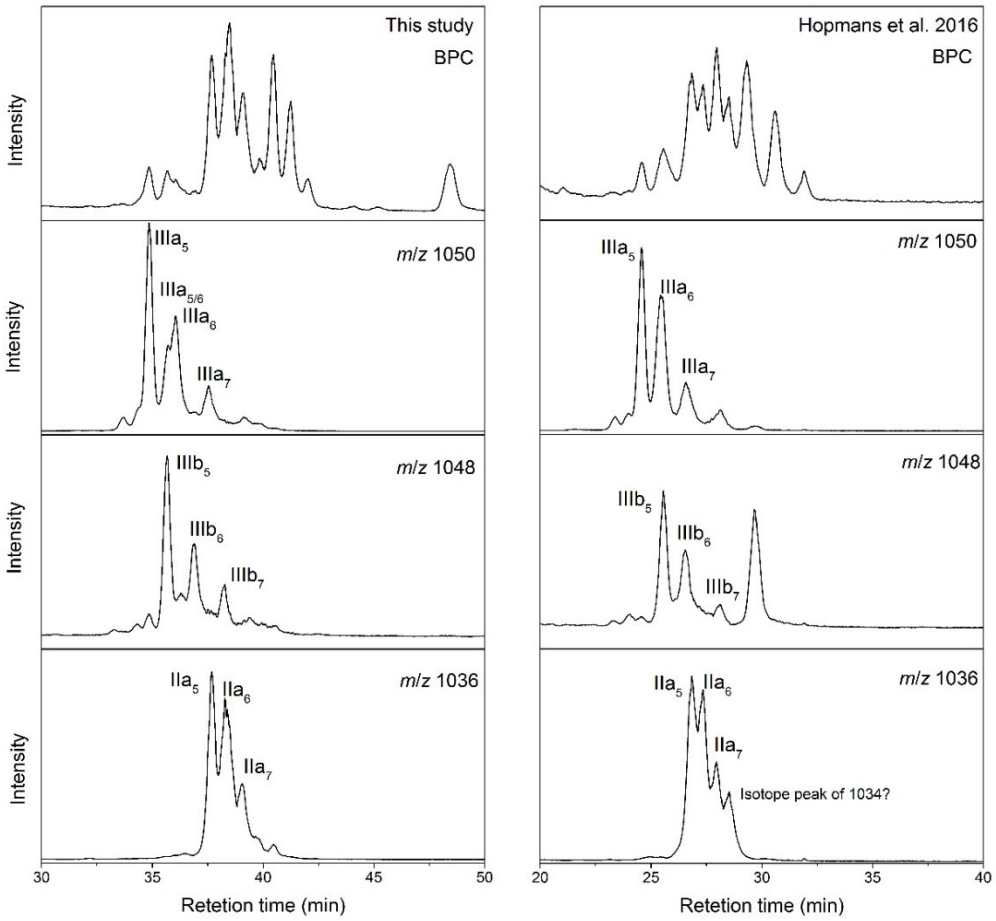


Fig. S2. HPLC–APCI-MS chromatograms of brGDGTs of lake sediment BSTH-D4 using the method presented in this study and the method reported by Hopmans et al. (2016), including base peak chromatogram (BPC), *m*/*z* 1050, *m*/*z* 1048 and *m*/*z* 1036 extracted ion chromatograms.

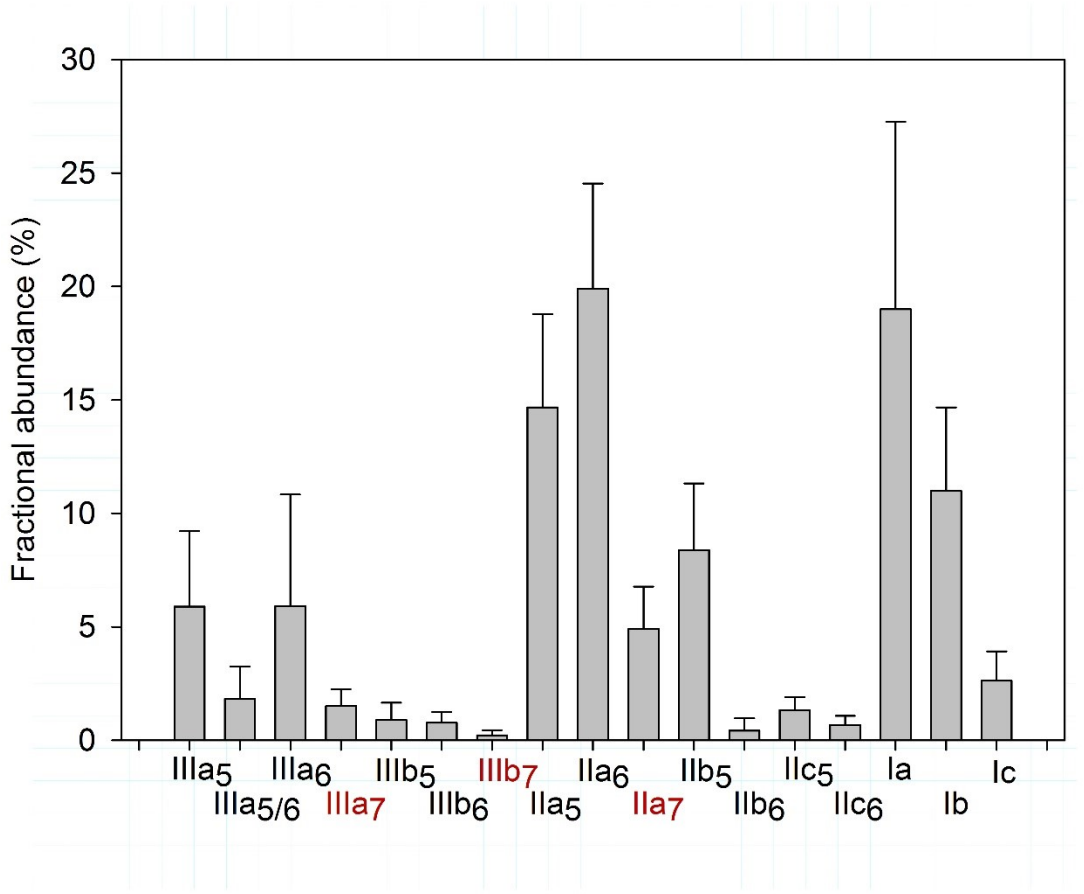


Fig. S3. Average fractional abundance of brGDGTs in the 102 Chinese lake sediments studied; error bar reflects ±1 SD.

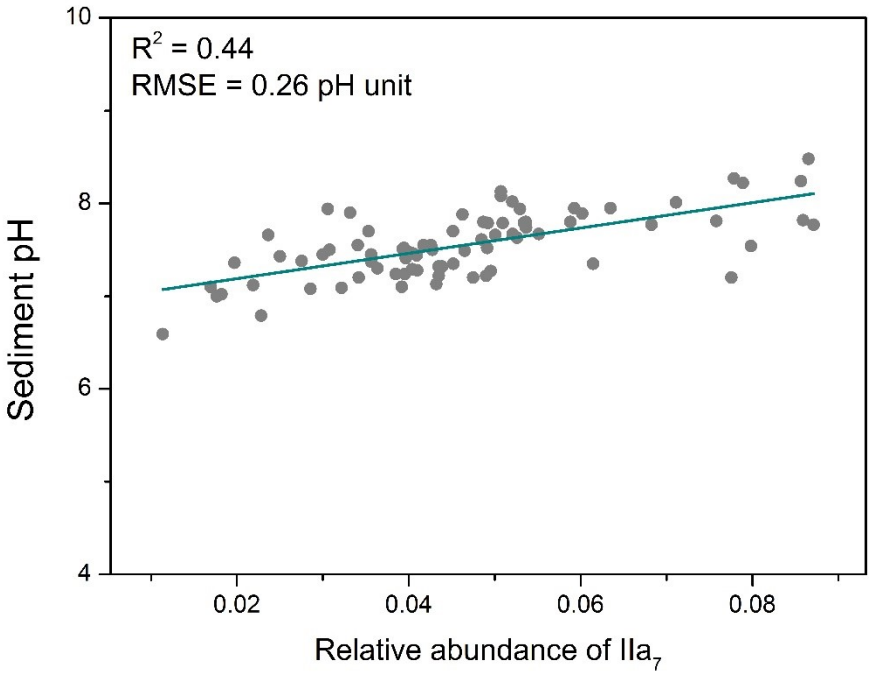


Fig. S4. Linear regression plot of the relative abundance of brGDGT-IIa$_7$ versus sediment pH in the Chinese lake sediments.

Table S1. Coordinates and environmental parameters (sediment pH, mean annual air temperature and mean annual precipitation) of Chinese lake sediments collected for novel brGDGT isomers identification (102 samples from 37 lakes) and the relative abundance of brGDGT-IIa$_7$ in all sediments.

Name	Lake	Longitude	Latitude	Relative abundance of IIa$_7$	sediment pH	MAT	MAP
DH D1	Dai Lake	112°40'9"	40°34'52"	0.08	8.2	5.6	381
DH D2	Dai Lake	112°40'42"	40°35'0"	0.09	8.5	5.6	381
WLSH D1	Ulansuhai Nur	108°50'24"	40°54'41"	n.d.	8.0	7.0	230
WLSH D2	Ulansuhai Nur	108°49'16"	40°53'47"	0.05	8.1	7.0	230
WLSH D3	Ulansuhai Nur	108°48'21"	40°51'53"	n.d.	8.0	7.1	233
WLSH D4	Ulansuhai Nur	108°49'53"	40°52'1"	0.06	7.9	7.1	233
HLH D1	Hu-Lun Lake	117°32'58.6"	49°11'57"	0.05	8.1	-0.5	295
HLH D2	Hu-Lun Lake	117°33'35.9"	49°13'15.6"	0.05	8.0	-0.6	295
HLH D3	Hu-Lun Lake	117°34'25.6"	49°14'16.2"	0.07	8.0	-0.6	296
HLH D4	Hu-Lun Lake	117°35'22.4"	49°15'25.1"	0.06	8.0	-0.6	296
HLH D5	Hu-Lun Lake	117°36'2.3"	49°16'17.5"	0.09	8.2	-0.7	298
HLH D7	Hu-Lun Lake	117°38'55.4"	49°17'4.2"	0.09	7.8	-0.7	299
HLH D8	Hu-Lun Lake	117°38'4.2"	49°18'16.8"	0.08	7.8	-0.7	299
LHH D1	Lianhuan Lake	124°10'14.3"	46°47'50"	0.03	7.9	4.0	415
LHH D2	Lianhuan Lake	124°9'26.4"	46°47'16.1"	0.07	n.a.	4.0	415
LHH D3	Lianhuan Lake	124°10'7.8"	46°46'30.9"	0.09	7.8	4.0	415
LHH D4	Lianhuan Lake	124°9'20.3"	46°46'18.3"	0.07	7.8	4.0	415
ZGH D1	Chagan Lake	124°15'18"	45°16'46"	0.05	7.9	5.1	431
ZGH D2	Chagan Lake	124°17'2"	45°14'9"	0.06	n.a.	5.1	435
ZGH D3	Chagan Lake	124°19'39"	45°13'9"	0.06	8.0	5.1	436
SHH D1	Songhua Lake	126°43'14.2"	43°42'17"	0.02	7.0	4.2	690
JPH D2	Jingpo Lake	128°59'18"	44°1'2"	0.01	n.a.	3.7	577
JPH D3	Jingpo Lake	128°59'57"	44°0'10"	0.02	n.a.	3.7	576

SXKH D1	Small Xinkai Lake	132°30'2.5"	45°21'3.9"	0.02	7.4	4.2	577
SXKH D2	Small Xinkai Lake	132°31'33.5"	45°20'52.0"	0.02	7.4	4.2	579
BXKH D1	Big Xinkai Lake	132°8'49.3"	45°17'57.3"	0.05	7.6	4.4	563
BXKH D2	Big Xinkai Lake	132°8'41"	45°17'8.6"	n.d.	7.5	4.4	562
BSTH D1	Bosten Lake	86°59'45"	41°56'9"	0.05	7.8	10.3	80
BSTH D2	Bosten Lake	86°59'50"	41°57'27"	0.05	7.8	10.2	81
BSTH D4	Bosten Lake	86°55'58"	41°59'14"	0.06	7.8	10.3	81
BSTH D5	Bosten Lake	86°54'22"	41°59'10"	0.05	7.8	10.3	81
BSTH D7	Bosten Lake	86°51'11"	41°55'11"	0.05	7.8	10.5	77
WLGH D1	Ulungur Lake	87°29'45"	47°15'36"	n.d.	8.0	5.8	132
WLGH D2	Ulungur Lake	87°26'29"	47°17'29"	0.04	7.7	5.8	134
TSTC D1	Heaven Lake of Tian Shan	88°7'40"	43°52'46"	n.d.	7.5	2.0	245
QHH D1	Qinghai Lake	100°31'49"	36°36'36"	0.02	n.a.	1.1	335
BYD D1	Baiyangdian Lake	115°56'25.3"	38°50'10.9"	0.08	8.3	12.8	487
BYD D2	Baiyangdian Lake	115°54'55"	38°50'27"	n.d.	n.a.	12.8	495
BYD D3	Baiyangdian Lake	116°00'22"	38°57'4"	0.04	7.3	12.8	495
BYD D4	Baiyangdian Lake	116°00'44"	38°56'58"	n.d.	n.a.	12.8	495
PYH D1	Poyang Lake	116°9'19"	29°42'10"	0.08	7.5	17.5	1474
PYH D2	Poyang Lake	116°12'28"	29°45'6"	0.02	6.8	17.5	1470
PYH D3	Poyang Lake	116°22'55"	29°3'42"	0.02	7.0	17.8	1433
PYH D4	Poyang Lake	116°19'32"	29°5'53"	0.01	6.6	17.8	1425
PYH D5	Poyang Lake	116°10'52"	29°13'38"	0.02	7.1	17.6	1506
PYH D6	Poyang Lake	116°4'59"	29°19'8"	0.02	7.1	17.6	1617
LH D1	Long lake	112°25'56"	30°59'19"	0.08	7.2	16.6	945
LH D2	Long lake	112°24'18"	30°25'30"	0.05	7.2	16.6	1079
LH D4	Long lake	112°21'38"	30°23'56"	0.05	7.3	16.6	1082
HH D1	Hong Lake	113°24'39"	29°54'17"	0.04	n.a.	16.9	1257
HH D2	Hong Lake	113°20'42"	29°50'25"	0.03	n.a.	17.0	1259
DC D1	Dian Lake	102°40'33"	24°54'16"	0.06	7.4	15.9	993

DC D2	Dian Lake	102°39'47"	24°56'5"	0.03	7.1	15.8	993
DC D5	Dian Lake	102°38'31"	24°58'55"	0.03	7.2	15.7	991
EH D1	Erhai Lake	100°10'57"	25°47'21"	0.02	7.7	15.7	1033
EH D3	Erhai Lake	100°11'12"	25°49'6"	0.04	7.5	15.7	1029
EH D5	Erhai Lake	100°10'53"	25°51'16"	0.04	7.4	15.7	1025
EH D7	Erhai Lake	100°10'12"	25°53'5"	0.04	7.5	15.7	1024
HFH D1	Red Maple Lake	106°25'17"	26°32'49"	0.04	7.5	15.9	1208
HFH D2	Red Maple Lake	106°24'45"	26°32'35"	0.03	7.1	15.8	1210
YLH D1	Yilong Lake	102°31'33"	23°40'54"	0.04	7.3	19.6	1045
TH D1	Tai Lake	120°0'22"	31°19'35"	0.04	7.5	15.7	1083
TH D2	Tai Lake	120°1'47"	31°19'12"	0.05	7.6	15.7	1081
TH D4	Tai Lake	120°4'50"	31°20'26"	0.05	7.8	15.7	1070
TH D6	Tai Lake	120°10'52"	31°25'35"	0.04	7.6	15.6	1038
TH D8	Tai Lake	120°11'32"	31°29'12"	0.05	7.7	15.6	1029
TH D10	Tai Lake	120°14'59"	31°23'2"	0.05	7.4	15.6	1029
XH D1	West Lake	120°8'23"	30°14'20"	0.05	7.5	16.8	1370
XH D2	West Lake	120°8'21"	30°14'9"	0.05	7.5	16.8	1374
LMH D1	Luoma Lake	118°13'10"	34°4'5"	0.03	7.9	14.6	739
LMH D3	Luoma Lake	118°15'9"	34°4'16"	0.04	7.3	14.6	744
LMH D4	Luoma Lake	118°14'59"	34°3'22"	0.05	7.2	14.6	745
HZH D1	Hongze Lake	118°40'5"	33°22'36"	0.05	7.7	14.9	844
HZH D2	Hongze Lake	118°38'25"	33°22'58"	0.05	7.7	14.9	839
HZH D3	Hongze Lake	118°31'20"	33°16'22"	0.05	7.9	15.0	822
HZH D4	Hongze Lake	118°28'24"	33°13'44"	0.05	7.8	15.1	818
HZH D5	Hongze Lake	118°42'15"	33°12'50"	0.05	7.7	15.0	848
HZH D6	Hongze Lake	118°44'34"	33°13'17"	0.05	7.7	15.0	839
WBH D1	Wabu Lake	116°53'20"	32°23'44"	0.06	7.7	15.8	843
WBH D4	Wabu Lake	116°53'47"	32°20'39"	0.04	7.4	15.8	853
CH D1	Chao Lake	117°40'26"	31°34'59"	0.04	n.a.	16.2	1049
CH D2	Chao Lake	117°36'40"	31°32'58"	0.04	7.2	16.3	1056

CH D3	Chao Lake	117°31'20"	31°31'15"	0.04	n.a.	16.3	1062
CH D4	Chao Lake	117°22'18"	31°37'16"	0.04	7.3	16.3	1010
XWH D1	Xuanwu Lake	118°47'28"	32°4'8"	0.04	7.3	15.6	998
XWH D2	Xuanwu Lake	118°47'38"	32°3'54"	0.04	7.2	15.6	1001
XWH D3	Xuanwu Lake	118°47'56"	32°4'4"	0.04	7.4	15.6	1002
DTH D1	Dongting Lake	112°42'48"	28°47'52"	0.04	7.5	17.5	1335
DTH D2	Dongting Lake	112°43'11"	28°46'50"	0.03	n.a.	17.5	1338
LGH D1	Longgan Lake	116°3'23"	29°56'19"	0.03	7.5	17.3	1404
LGH D2	Longgan Lake	116°3'41"	29°56'33"	0.03	7.5	17.1	1401
LZH D1	Liangzi Lake	114°35'19"	30°18'4"	0.04	7.1	17.3	1318
LZH D2	Liangzi Lake	114°33'8"	30°15'47"	0.04	7.1	17.3	1323
DL D1	East Lake (Wuhan)	114°22'3"	30°32'47"	n.d.	n.a.	n.a.	n.a.
DL D3	East Lake (Wuhan)	114°22'58"	30°33'34"	n.d.	n.a.	n.a.	n.a.
ZYH D1	Zhaoyang Lake	116°56'55"	34°54'55"	0.03	7.6	14.5	724
ZYH D2	Zhaoyang Lake	116°54'30"	34°54'23"	0.04	7.5	14.5	723
WSH D1	Weishan Lake	117°11'30"	34°39'25"	0.04	7.6	14.6	753
WSH D2	Weishan Lake	117°9'2"	34°43'59"	n.d.	7.5	14.6	751
DPH D1	Dongping Lake	116°12'41"	35°58'43"	0.05	7.5	14.0	615
DPH D2	Dongping Lake	116°13'42"	35°58'44"	0.03	7.4	14.0	615
DPH D4	Dongping Lake	116°14'22"	35°58'4"	0.04	7.2	14.0	618

n.d. means not detected

n.a. means not available

3. In situ production of core and intact bacterial and archaeal tetraether lipids in groundwater

Su Ding, Bernd Kohlhepp, Susan Trumbore, Kirsten Küsel, Kai-Uwe Totsche, Georg Pohnert, Gerd Gleixner, Valérie F. Schwab

Published in *Organic Geochemistry* (2018), 126, 1-12

Manuscript II

Statement on individual contributions of doctoral candidate in a cumulative doctoral thesis

Publication (complete citation): **Ding, S.**[1], Kohlhepp, B.[2], Trumbore, S.[3], Küsel, K.[4], Totsche, K.U.[5], Pohnert, G.[6], Gleixner, G.[7], Schwab, V.F.[8], 2018. In-situ production of core and intact bacterial and archaeal tetraether lipids in the pristine groundwater. *Organic Geochemistry* 126, 1-12.								
Involved in	Author number							
	1	2	3	4	5	6	7	8
Conception of research approach	X					X	X	X
Planning of research activities	X						X	X
Data collection	X	X						
Data analysis and interpretation	X	X	X	X	X	X	X	X
Writing a manuscript	X						X	X
Suggested publication equivalence value to be	1.0							

The subsequent manuscript has not been used in any other cumulative PhD thesis. Reprint permissions for this publication were obtained from the publisher and the coauthors.

Organic Geochemistry 126 (2018) 1–12

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

In situ production of core and intact bacterial and archaeal tetraether lipids in groundwater

Su Ding [a,*], Bernd Kohlhepp [b], Susan Trumbore [a], Kirsten Küsel [c,d], Kai-Uwe Totsche [b], Georg Pohnert [e,f], Gerd Gleixner [a], Valérie F. Schwab [a,e]

[a] Max Planck Institute for Biogeochemistry, Hans-Knöll-Str. 10, 07745 Jena, Germany
[b] Department of Hydrogeology, Institute of Geosciences, Friedrich Schiller University Jena, Burgweg 11, 07749 Jena, Germany
[c] Department of Aquatic Geomicrobiology, Institute of Ecology, Friedrich Schiller University Jena, Jena, Germany
[d] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
[e] Institute for Inorganic and Analytical Chemistry, Bioorganic Analytics, Department of Chemistry and Earth Sciences, Friedrich-Schiller-Universität, Lessingstr. 8, 07743 Jena, Germany
[f] Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany

ARTICLE INFO

Article history:
Received 7 December 2017
Received in revised form 28 September 2018
Accepted 11 October 2018
Available online 15 October 2018

Keywords:
Core and intact polar GDGTs
Karstic groundwater
Source trace
Microbial community

ABSTRACT

Groundwater ecosystems host diverse and complex microbial communities that play important roles in the biogeochemical processing of organic matter and in the maintenance of drinking water quality. Here we investigated the microbial community in suspended particulate matter (SPM) of biogeochemically distinct groundwaters (Hainich Critical Zone Exploratory) by analyzing branched and isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) from bacteria and archaea, respectively. The contributions of those lipids derived from dead and living organisms were determined by analyses of the core lipid distributions of core and intact polar GDGTs. We compared the groundwater GDGT distributions to the ones from soils of potential recharge areas and with archaeal 16S rRNA-gene based community reconstructions to estimate their origin in these terrestrial subsurface environments and thus their potential use for evaluating soil inputs into groundwater. In soils, the relative abundance of intact polar branched GDGTs (brGDGTs) was lower than that of isoprenoid GDGTs (isoGDGTs; 2% vs 5% of total GDGTs), while the opposite trend (71% vs 22% of total GDGTs) was observed in the core lipid pools. This supports previous observations that soil brGDGT-producing bacteria might be more active and thus have higher regeneration rates than the isoGDGT-producing archaea. We found similar trends in the groundwater that might indicate higher activity (i.e., cell division) of brGDGT-producing bacteria than of isoGDGT-producing archaea. The higher relative abundance of the hexamethylated brGDGT in the groundwater SPM (mean 65 ± 9%, n = 5) than in soils (mean 16 ± 7%, n = 22) indicated an in situ origin of brGDGT-producing bacteria. Higher contributions of penta- and tetra-methylated brGDGTs, which suggested some inputs from soil bacteria, was only detected in two out of seven groundwater samples. The strong correlation between core and intact polar isoGDGTs (R^2 = 0.99, n = 7) in groundwater SPM indicated low disturbance (e.g., surface inputs) and suggested indigenous archaeal communities in the groundwater. This was supported by the results from a previous 16S rRNA-gene study that detected distinct archaeal groups in soils and groundwater. This first GDGT study in groundwater demonstrated that even dynamic karstic subsurface environments host an indigenous bacterial and archaeal community that is adapted to the living conditions. Furthermore, fast recharge events are likely detectable using tetraether lipids from the soil microbial community.

1. Introduction

Groundwater is the largest freshwater reservoir in the liquid water cycle, and not only plays an important role for sustaining human life, but also provides a habitat for an impressive number of microbial species (Danielopol et al., 2003). Among the microbes in groundwaters, bacteria are expected to be most abundant, representing over 70% of the total cell count (Zinger et al., 2012), while archaea are expected to account for a relatively small (2–20% of the total cell count) fraction (Detmers et al., 2004). Bacteria play key roles that influence groundwater quality and organic matter degradation (Goldscheider et al., 2006), while their metabolisms appear somehow linked to hydrogeochemical conditions of the aquifers

* Corresponding author.
E-mail address: sding@bgc-jena.mpg.de (S. Ding).

https://doi.org/10.1016/j.orggeochem.2018.10.005
0146-6380/

and particularly to surface-subsurface relationships (e.g., Akob and Küsel, 2011; Schwab et al., 2017). Archaea have been studied fairly intensively in the marine subsurface (e.g., Teske and Sorensen, 2008), but very little is known about archaeal communities in terrestrial groundwaters (Lazar et al., 2017). In such remote environments, archaea are expected to play an important ecological function, because they have evolved a variety of energy metabolisms using organic and/or inorganic electron donors and acceptors (Biddle et al., 2006). For instance, in groundwaters where surface organic matter is limited, methanogenic archaea can produce methane from sedimentary rocks while generating the energy needed to sustain microbial life and also re-activating buried organic matter in subsurface environments (Simkus et al., 2016).

Our knowledge about the microorganisms in groundwaters is largely based on extractable 16S rRNA and DNA (Griebler and Lueders, 2009). Despite the superiority of these molecular approaches for the analysis of microbial community compositions, lipid-based methods, such as the analysis of phospholipid-derived fatty acids (PLFAs), have the advantage of enabling discernment of the physiological changes of the bacterial communities in relation to environmental conditions (Martino et al., 1998; Green and Scow, 2000; Schwab et al., 2017). Glycerol dialkyl glycerol tetraethers (GDGTs), which are investigated in this study, have been associated with both bacterial and archaeal groups. Isoprenoid GDGTs (isoGDGTs) are archaeal membrane lipids containing two C_{40} isoprenoid (biphytanyl) chains that are ether-linked to two glycerols (Kates, 1993; Koga and Morii, 2005). They are likely synthesized by thermophilic archaea and some mesophilic methanogenic Euryarchaeota, as well as by Thaumarchaeota (Koga and Morii, 2005; Pitcher et al., 2010, 2011; Schouten et al., 2012; Elling et al., 2017). Another important class of tetraether membrane lipids, are branched GDGTs (brGDGTs). They are produced by unknown bacteria (Anderson et al., 2014; Buckles et al., 2014; Zell et al., 2014), although they have been found in some Acidobacteria (Weijers et al., 2009; Sinninghe Damsté et al., 2011).

A technology has been intensively developed by which GDGTs can be as environmental proxies and they are widely applied to soil, marine and lake environments (Schouten et al., 2002; Sinninghe Damsté et al., 2002; Weijers et al., 2007; Tierney and Russell, 2009; Günther et al., 2014; Wu et al., 2014). However, heretofore, GDGTs from groundwater have never been analyzed. In speleothems, Blyth et al. (2014) found different brGDGT distributions associated with soil bacteria, but their potential for use to infer surface soil input into the groundwater system is still poorly understood. GDGTs can be analyzed using two different techniques.

Core lipids (i.e., the alkyl chain linked by the ether bond to the original glycerols: the core GDGTs) are assumed to represent fossil biomass, and in a depositional environment, could accumulate in soils or sediments over millions of years (Schouten et al., 2013). In contrast, the intact polar lipids likely represent living bacterial and archaeal biomass (Sturt et al., 2004; Liu et al., 2011; Sinninghe Damsté et al., 2011), because they degrade rapidly upon cell lysis (Harvey et al., 1986). The relationships between the abundance of core and intact polar GDGTs can be used to estimate sources and storage of those specific lipids (Ingalls et al., 2012; French et al., 2015) and thus provide a link to the activities of isoGDGT-producing archaea and brGDGT-producing bacteria in subsurface environments. Previous studies of archaea have shown that intact polar isoGDGTs with glycosylic head groups were predominant in deep marine sediments (Lipp et al., 2008; Lipp and Hinrichs, 2009), suggesting that archaea contribute substantially to the subsurface biosphere. However, there is still considerable debate as to what extent archaeal IPLs represent living vs fossil biomass in subsurface regions (Schouten et al., 2010; Logemann et al., 2011; Xie et al., 2013).

In the work reported here, we investigated branched and isoprenoid GDGTs (the core lipid distributions of core and intact polar GDGTs) in two superimposed carbonate-rock aquifer assemblages, as well as in soils of preferential groundwater recharge areas. Samples were taken along a monitored groundwater well transect of the Hainich Critical Zone Exploratory (Hainich CZE; central Germany) of the AquaDiva Collaborative Research Centre (CRC: Küsel et al., 2016; Kohlhepp et al., 2017). Previous metaproteomics studies involving 16S and 18S ribosomal RNA gene and phospholipid fatty acid analyses of the groundwater SPM, detected various protists (flagellates, ciliates, and amoeba), fungi, bacteria, and archaea (Risse-Buhl et al., 2013; Opitz et al., 2014; Herrmann et al., 2015; Nawaz et al., 2016; Herrmann et al., 2017; Starke et al., 2017, Schwab et al., 2017; Nowak et al., 2017). Quantitative PCR using 16S rRNA genes across all sites along the Hainich CZE transect indicated that archaea represents 0.03–8.2% of the total microbial population (Küsel et al., 2016). In this study, we aimed to: (1) evaluate the relationship of bacterial and archaeal GDGTs in Hainich soils and groundwaters; (2) determine the source and distribution of GDGTs in groundwater suspended particulate matter; and (3) give first assessments of isoGDGT-producing archaea in the groundwater and to compare them to published results from archaeal 16S rRNA genes community reconstructions (Lazar et al., 2017).

2. Study area

The Hainich CZE covers 430 km^2 of the northwestern part of the Thuringian Basin in central Germany (Küsel et al., 2016). The groundwater transect includes five well sites (H1 to H5) and spans a distance of 5.4 km from the summit (H1) to the footslope area (H5) along the eastern hillslope of the Hainich low mountain range (Fig. 1). Each well site comprises up to three groundwater wells that access two aquifer assemblages of the Upper Muschelkalk group, which is characterized by alternated bedding of limestone (aquifer) and marlstone (aquitard) (Fig. 2). The types of land use grade from deciduous forest (summit to midslope) to cropland and pasture (footslope). Because the strata dip steeper than the slope angle, geological formations that are located deeper in the stratigraphy, outcrop at higher slope positions. For this reason, aquifer outcrop areas represent preferential infiltration zones (Fig. 2; Kohlhepp et al., 2017). The lower aquifer assemblage (HTL, Hainich transect lower aquifer assemblage; Küsel et al., 2016), includes one aquifer that is sampled between the recharge area in the summit position (H11 at 6 m depth) and the discharge area (H51 at 88 m depth). The geological formation hosting the HTL is mainly formed by the Trochitenkalk formation (moTK, Fig. 2; Table 1) characterized by moderate karstification. This and thin soil cover at the aquifer outcrop (summit of the transect) allow rapid infiltration flow and transport, which favor oxic to suboxic groundwater conditions until reaching the discharge area (DO of ca. 3.7 mg/L in H41 and 3.1 mg/L in H51; Küsel et al., 2016). The Hainich transect upper aquifer (HTU) is hosted in the Meissner formation (moM) and includes nine laterally continuous aquifer storeys of the fracture aquifer type (moM-1 to moM-9, Fig. 2). These are vertically separated by marlstone aquitard intervals. Capture zones of the HTU are characterized by mixed forest, crop or grassland land use, and relatively thick soils with low hydraulic conductivities. These conditions, in combination with narrow fractures (acting as groundwater flow paths) of the aquitards, result in low redox potential, O_2 and organic carbon consumption, and chemical equilibration of the groundwater with the bedrock components (Küsel et al., 2016, Nowak et al., 2017; Kohlhepp et al., 2017). As a result, average concentrations of dissolved oxygen (DO) and total organic carbon (TOC) in the HTU groundwater range from ca.

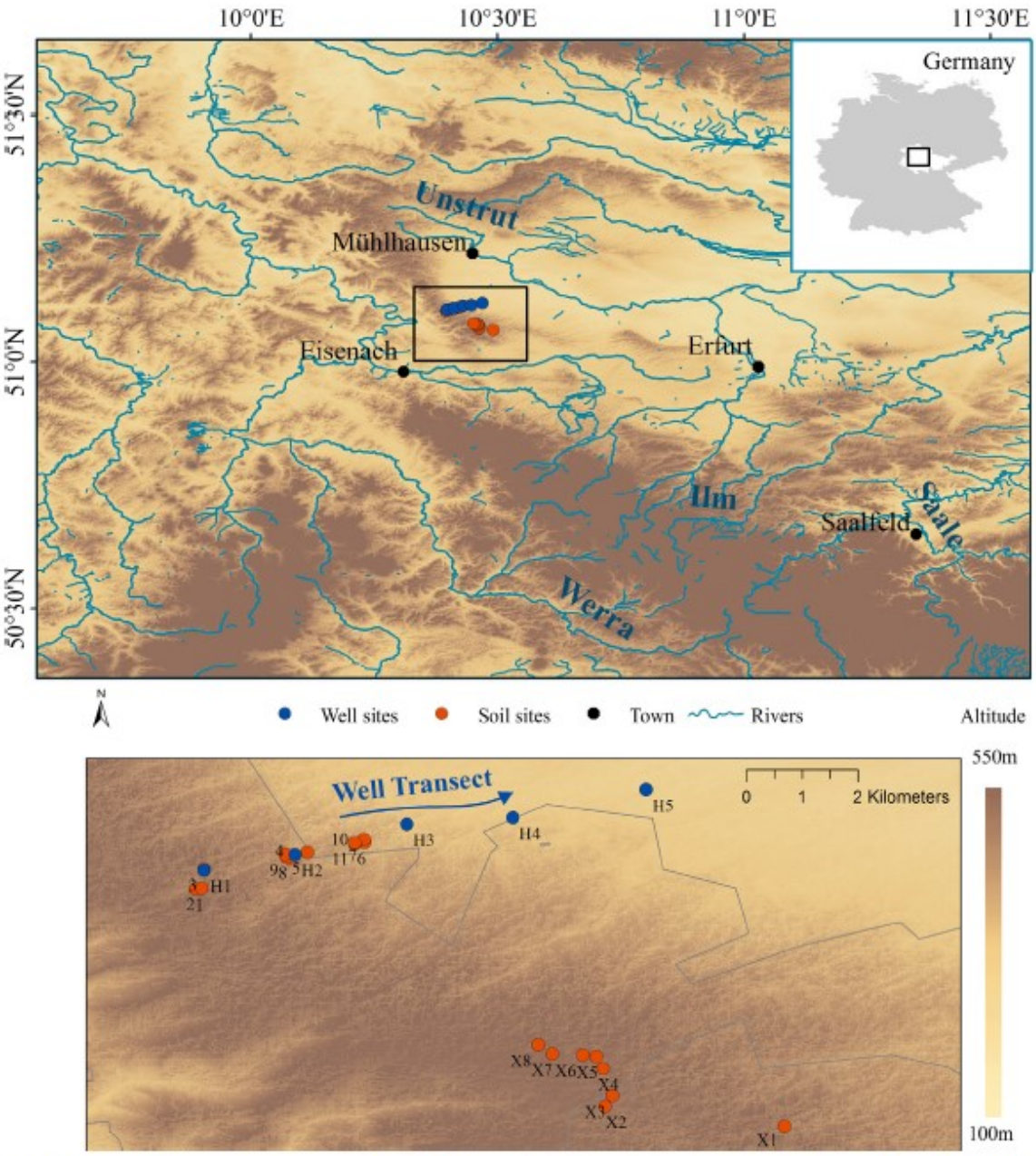


Fig. 1. Map of sample locations at the Hainich Critical Zone Exploratory in western Thuringia (Germany; modified from Küsel et al., 2016) and detailed section of the study area showing the sampling stations and the AquaDiva groundwater well transect. Solid dots with different colors represent different sample types: aquifer SPM (blue), surface soils (orange). The inset map is Germany, and the white shape within is Thuringia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.6 mg/L and 0.09% in H32 (proximal to fracture zones in the aquifer outcrop) to 0 mg/L and approximately 0.05% in wells H42/43/53 located downhill in the aquifer discharge area. The small Eichbach River runs through sample sites H4 and H5. Further details about the geological and stratigraphic settings and well monitoring of the Hainich CZE can be found in Küsel et al. (2016) and Kohlhepp et al. (2017).

3. Materials and methods

3.1. Sampling

In August 2015, suspended particulate matter (SPM) from both aquifer assemblages were collected from locations H3 (well H32), H4 (wells H41, H42, and H43), and H5 (wells H51, H52, and H53) during a regular sampling campaign within the coordinated joint monitoring program of the CRC (Campaign 66, Fig. 1; Table 1). Groundwater (35–500 L) was sampled using a submersible pump (Grundfos SQ5-70, Grundfos, Denmark) and filtered on site with 0.3 μm pore size pre-combusted (5 h at 500 °C) glass fiber filters (Sterlitech, USA). Filters with 0.3 μm pore size were used as it has been shown that cultured Thaumarchaeota have a straight rod shape with a diameter of 0.17–0.22 μm and a length of 0.5–0.9 μm (Könneke et al., 2005). More details about SPM sampling can be found in Schwab et al. (2017). The filters with SPM were immediately frozen over dry ice and then transported to the lab where they were stored at −80 °C until further treatment.

The soil sampling sites were selected at locations in the preferential recharge areas of the 10 aquifer storeys (Figs. 1 and 2). Their

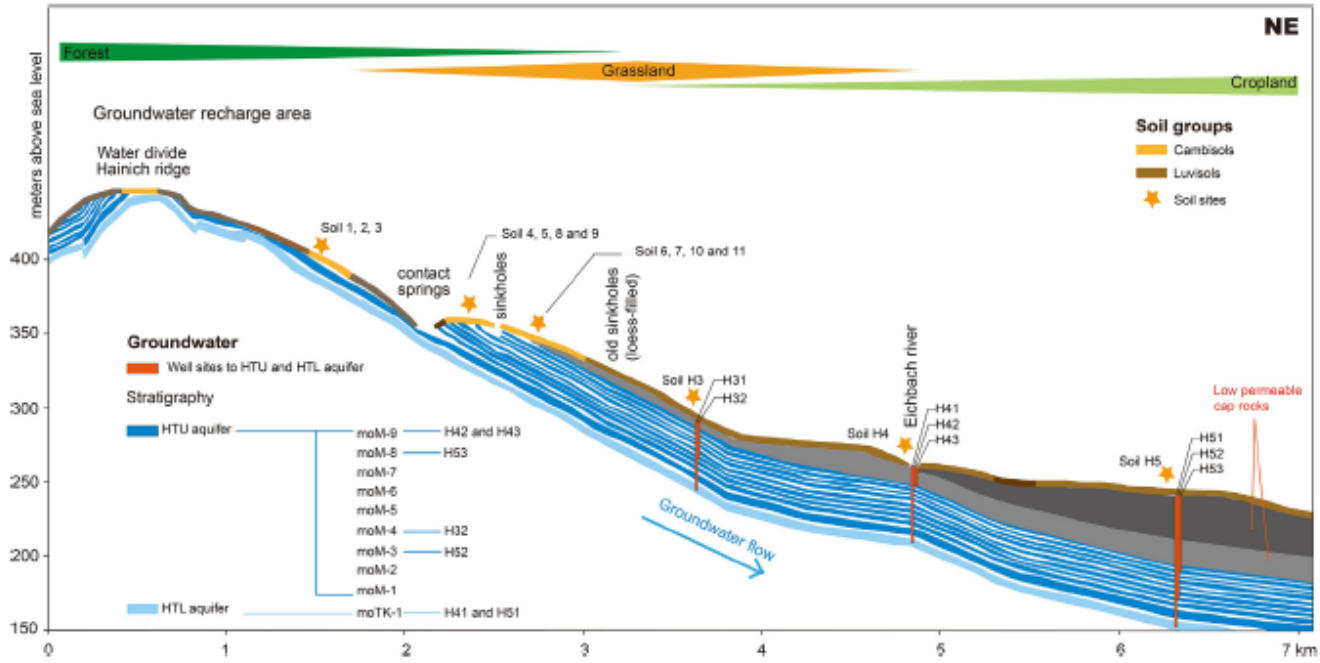


Fig. 2. Geological cross section of the Hainich Critical Zone Exploratory showing the stratified alternation of aquifers and aquitards accessed by groundwater wells at five sites (modified from Kohlhepp et al., 2017). The most important soil groups (cambisols and luvisols), land use types, and sampling sites of this study are shown as well.

Table 1
Location and bulk data for the aquifers and soils collected from the Hainich Critical Zone Exploratory.

	No.	Vegetation/aquifer type	Latitude (°E)	Longitude (°N)	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	TOC (%)
Aquifers	H32	Upper aquifer	51.114	10.431	7.3	2.6	10.2	0.09
	H42	Upper aquifer	51.115	10.448	7.2	0.0	9.8	0.03
	H43	Upper aquifer	51.115	10.448	7.2	0.0	9.7	0.06
	H52	Upper aquifer	51.119	10.469	7.3	0.0	10.6	0.15
	H53	Upper aquifer	51.119	10.469	7.3	0.0	11.6	0.05
	H41	Lower aquifer	51.115	10.448	7.2	3.7	10.2	0.04
	H51	Lower aquifer	51.119	10.469	7.1	3.0	10.6	0.06
Soils	1	Grassland	51.103	10.397	5.3	n.m.	n.m.	3.76
	5	Grassland	51.109	10.415	5.4	n.m.	n.m.	2.32
	6	Grassland	51.111	10.424	6.5	n.m.	n.m.	4.39
	10	Grassland	51.111	10.422	6.7	n.m.	n.m.	6.58
	X1	Grassland	51.065	10.492	7.2	n.m.	n.m.	3.42
	X2	Grassland	51.068	10.463	7.0	n.m.	n.m.	4.81
	X3	Grassland	51.070	10.464	6.7	n.m.	n.m.	4.81
	X4	Grassland	51.074	10.462	5.1	n.m.	n.m.	3.39
	X5	Grassland	51.076	10.461	6.1	n.m.	n.m.	5.04
	H4	Grassland	51.115	10.448	7.1	n.m.	n.m.	4.18
	2	Forest	51.104	10.398	4.5	n.m.	n.m.	2.89
	3	Forest	51.107	10.398	4.7	n.m.	n.m.	2.14
	4	Forest	51.108	10.412	6.0	n.m.	n.m.	4.01
	8	Forest	51.109	10.411	5.0	n.m.	n.m.	3.00
	9	Forest	51.109	10.411	4.9	n.m.	n.m.	3.07
	X6	Forest	51.077	10.459	5.9	n.m.	n.m.	4.56
	X7	Forest	51.077	10.454	6.4	n.m.	n.m.	5.00
	X8	Forest	51.078	10.452	6.3	n.m.	n.m.	6.32
	7	Cropland	51.111	10.424	5.2	n.m.	n.m.	1.98
	11	Cropland	51.111	10.422	7.2	n.m.	n.m.	2.32
	H3	Cropland	51.114	10.431	7.3	n.m.	n.m.	2.37
	H5	Cropland	51.119	10.469	7.4	n.m.	n.m.	1.57

n.m. = not measured.

major land uses are forest (summit/shoulder region, unmanaged and managed deciduous forest: sites 2 to 4, 8, 9, H1, and H2), grassland (midslope: sites 1, 5, 6, 10, H3, and H4), and cropland (footslope: sites 7, 10, H4, and H5). Soil samples (numbered 1–11) were collected along the assessed groundwater flow path. Soil samples labeled H3, H4, and H5 were collected in close proximity to the well sites to evaluate potential vertical permeation (Fig. 2). In addition, eight soil samples (named X1–X8) from the summit and shoulder region within the Hainich national park were collected 5 km from the AquaDiva well transect (Fig. 1) to assess lateral groundwater flow parallel to the slope direction. Samples of topsoil (0–10 cm) were taken within a 5 m^2 square after removing the litter layer. To reduce spatial heterogeneity, five points were sampled in each sampling square: four points (one each) at

the corners and one point in the center (Nambiar et al., 2004). The soils were stored at −20 °C until analysis.

3.2. Lipid extraction and GDGT measurements

In this study, we used sonic extraction instead of the commonly used modified Bligh and Dyer method (Sturt et al., 2004; Oba et al., 2006) because it showed higher extraction efficiency of intact polar GDGTs (Huguet et al., 2010). Intact polar GDGT concentrations were quantified using the common chromatography separation method with silica gel (Schouten et al., 2012). In detail, freeze-dried filters and surface soils (~8 g) were extracted using methanol (20 mL), methanol/dichloromethane (1:1, v/v, 20 mL), and dichloromethane (20 mL) using ultrasound. The combined extracts were concentrated to near dryness using a rotary evaporator and transferred to small vials. The separation of core and intact polar GDGTs was achieved using silica gel chromatography and the procedure developed by Oba et al. (2006) and Pitcher et al. (2009), and further modified by Tierney et al. (2012). In brief, the extracts were divided into two fractions by elution with hexane/ethyl acetate (1:1, v/v, 4 mL) and methanol (8 mL) through a silica column (0.9 g, 230–400 mesh). The nonpolar fraction (core lipids) was dried under N_2 and 0.12 μg of C_{46} glycerol tribiphytanyl glycerol tetraether (GTGT) standard (Huguet et al., 2006) was added. Before GDGT analysis, the fraction was dissolved in 300 μL hexane/ethyl acetate (84:16, v/v) and filtered through a 0.45 μm PTFE filter (Multoclear-13). The intact polar GDGTs in the dried polar fraction were hydrolyzed to core lipids by 5% HCL: methanol at 70 °C for 4 h (French et al., 2015). After the acid hydrolysis, 1 N KOH in methanol (96%) and MilliQ water were added to achieve a 1:1 ratio of methanol/water (v/v; Tierney et al., 2012). The mixture was extracted three times with dichloromethane. The dichloromethane extract was dried under N_2 and treated the same as the nonpolar fraction before being analyzed for GDGTs.

Branched and isoprenoid GDGTs were analyzed following the method of Ding et al. (2016), using an Agilent 1200 series HPLC system coupled to a Thermo Scientific Orbitrap Velos Pro ion trap mass spectrometer (HPLC–MS) equipped with an autoinjector and Xcalibur™ software. Two Hypersil GOLD silica columns (150 mm × 2.1 mm; 1.9 μm; Thermo Fisher Scientific, USA) in tandem were used for GDGT separation (Yang et al., 2015; Ding et al., 2016). The injected samples were eluted isocratically with a solvent mixture of 84% *n*-hexane and 16% ethyl acetate for 65 min (0.2 mL/min), followed by 20 min of 100% ethyl acetate as backflush. Subsequently, the system was equilibrated for 30 min at the initial condition. Atmospheric pressure chemical ionization (APCI) conditions were set according to De Jonge et al. (2013). Spectra were recorded in selected ion monitoring (SIM) mode in the mass range of *m/z* 1017–1051, 1291–1303, and 744. Concentrations of GDGTs were determined by comparing their peak areas with the internal standard (Huguet et al., 2006) and an equal response factor for all GDGTs and the internal standard was assumed.

3.3. pH and element parameters

The measurement of soil sample pH was carried out according to Ding et al. (2015). Soil material was mixed with MilliQ water in a ratio of 1:2.5 (g/mL) and shaken in an overhead shaker for 1 h. Then it was measured three times using a pH meter (WTW pH 538 pH/mV-Meter). For each soil sample, pH is given as the arithmetic mean of three duplicate measurements with a standard deviation of ± 0.05. For groundwater, the environmental parameters (temperature, pH, DO, and redox potential) were measured during the collection using a flow-through chamber equipped with a probe (FDO 925) for dissolved oxygen and a multi-parameter meter (Multi 3430 IDS, WTW GmbH, Germany). The TOC of the groundwater SPM and soil were measured after 12 h treatment with 8% H_2SO_3 and 1 N HCL solution separately; then analyzed using a Vario EL II Element Analyzer (Verardo et al., 1990).

3.4. Statistical methods

To determine the source of GDGTs in groundwater, comparisons of the concentrations of core and intact polar GDGTs in both surface soils and aquifer SPM were done using one-way ANOVA followed by a post hoc Bonferroni test.

4. Results

4.1. Relations between core and intact polar GDGTs in the groundwater and soils of recharge areas

Because the concentration of newly identified 7-methyl brGDGT isomers, previously found in lake sediments (Ding et al., 2016), was below 1% of the total brGDGTs, only the more abundant 5- and 6-methyl brGDGT isomers are reported here. In the core lipid pool of soils, the total concentration of brGDGTs ranged from 2.91 to 25.0 μg/g TOC (mean 7.76 μg/g TOC), while core isoGDGT concentrations ranged from 0.02 to 14.8 μg/g TOC (mean 2.87 μg/g TOC; Fig. 3, Table 2). The concentrations of core brGDGTs in groundwater SPM ranged from 0.07 to 1.28 μg/g TOC (mean 0.55 μg/g TOC) and the core isoGDGT concentration ranged from 0.03 to 0.54 μg/g TOC (mean 0.20 μg/g TOC).

In the intact polar lipid pool of the soil, the total concentration of brGDGTs varied between 0.05 and 0.54 μg/g TOC (mean 0.22 μg/g TOC) and the isoGDGT concentrations varied between 0.003 and 2.14 μg/g TOC (mean 0.28 μg/g TOC; Fig. 3, Table 2). In groundwater SPM, the brGDGT concentrations ranged from 0.01 to 0.22 μg/g TOC (mean 0.11 μg/g TOC) and the isoGDGT concentrations ranged from 0.03 to 0.26 μg/g TOC (mean 0.10 μg/g TOC).

Large variances in the relative abundance of the total intact polar brGDGTs and isoGDGTs, and of total core brGDGTs and isoGDGTs, were observed between the soils of different vegetation types and in groundwater (Fig. 4). Forest soils had the lowest core isoGDGT relative abundance (mean 5 ± 4%, n = 8), whereas croplands had the highest (mean 53 ± 13%, n = 4). The relative abundance of intermediate core isoGDGTs was measured in grassland (mean 28 ± 21%, n = 10). In groundwater, the mean relative abundance of the core isoGDGTs was 30 ± 24% (n = 7). In addition, the relative abundances of the isoGDGTs and brGDGTs in both core and intact polar lipid fractions of soil (n = 22) and groundwater SPM (n = 7) samples are shown in Supplementary Fig. S2.

4.2. Composition of core branched GDGTs in aquifers and soils

The composition of the core brGDGTs in soils and aquifers, representing fractional (relative) abundances of the summed tetra-, penta-, and *hexa*-methylated branched GDGTs as end-members (Sinninghe Damsté, 2016), showed that the soils had compositions like those reported for mid-latitude global soils. These were characterized by the predominance of pentamethylated brGDGTs (mean 53%, n = 22, Fig. 5). The results from the groundwater samples H32 and H53 plotted close to those of the topsoil samples X1–X3, as well as H3 and H5 (Fig. 5). H32 had a higher tetramethylated brGDGT percentage and its results yielded a plot similar to those with the Yenisei delta and the Kara sea sediments. In contrast, H53 had a higher hexamethylated brGDGTs percentage, which was plotted at the lower edge of the global soils (Fig. 5). The other groundwater SPM measures (H41, H42, H43, H51, and H52) deviated from the trend of global soils and marine sediments, and their

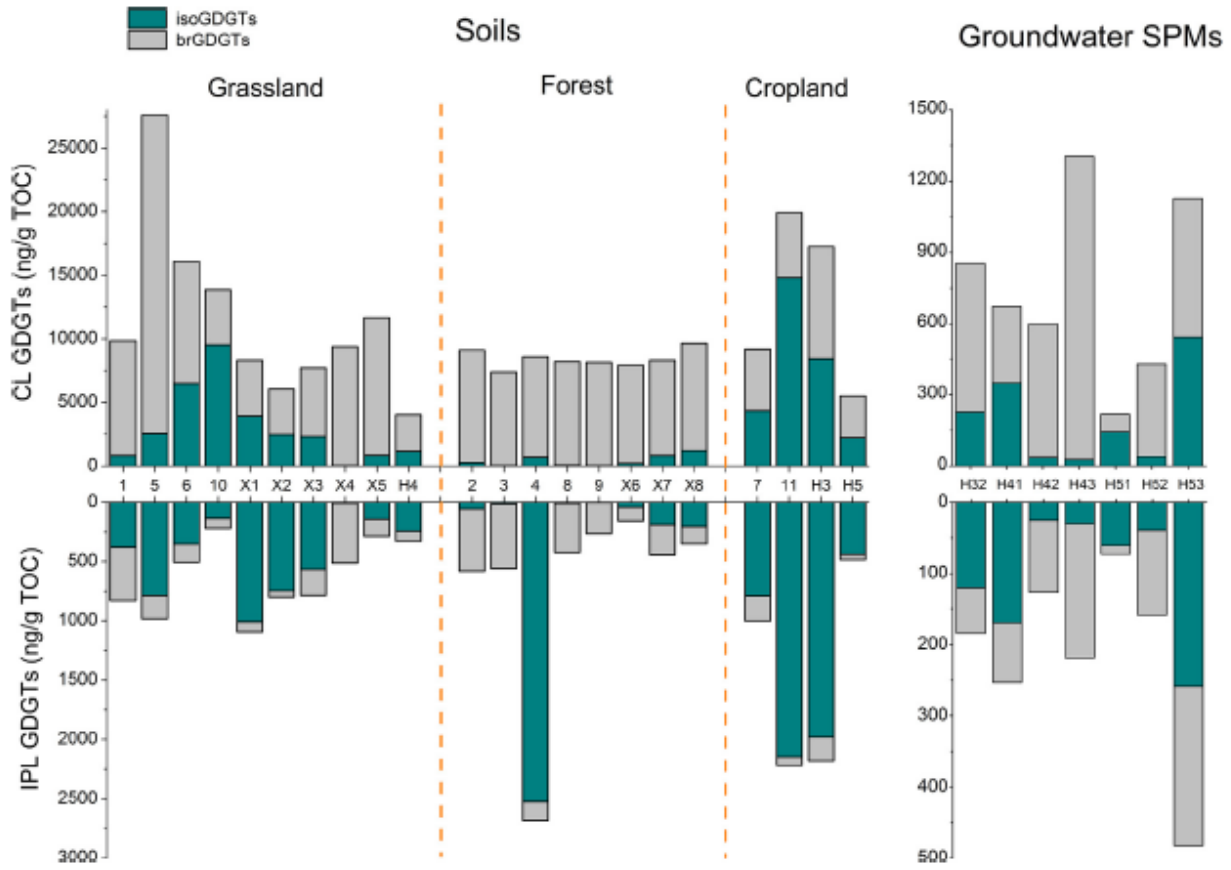


Fig. 3. Bar plots showing the summed absolute abundances (μg/g TOC) of brGDGTs and isoGDGTs (in the form of core and intact polar fractions) in both surface soils (left) and aquifer SPM (right). Soils are separated into three groups by different vegetation types (grassland, forest, and cropland). Groundwater samples are plotted on a different scale than the soil samples.

abundance showed a unique distribution with predominantly hexamethylated brGDGTs (mean 65 ± 9%, n = 5).

5. Discussion

5.1. Different relationships of core and intact polar GDGTs in soils and groundwater

In groundwater, the average absolute abundance of core and intact polar GDGTs was respectively 14 and 4 times lower than in the soils of the recharge areas (Fig. 3 and Supplementary Fig. S1). Typically, sediments from rivers, estuaries, and sand ponds have concentrations of intact polar and core GDGTs (brGDGTs + isoGDGTs) either comparable to, or up to an order of magnitude higher than, those in surrounding soils (Tierney et al., 2012; French et al., 2015). Commonly, the sources of intact polar GDGTs are biomass production, either by bacteria or archaea, whereas the sink includes the hydrolysis of polar head groups after cell lysis. In soils, hydrolyzed lipids directly increase the fossil pools of the core lipids. However, in groundwater, transport may include an additional source or sink of core GDGTs either by input from allochthonous sources, such as soils from the recharge areas, or by transfer from transported particles. Very low GDGT abundance in groundwater may thus point to both low allochthonous inputs and low microbial activity as is commonly observed in subsurface environments (Griebler and Lueders, 2009). In such environments, scarcity of nutrient and energy sources forces microbial communities to reduce their activities and forces individuals to disperse (Jørgensen and Marshall, 2016). This would result in a low turnover rate of the organic matter as the remaining lipids would not be subjected to microbial degradation (Schmidt et al., 2011).

In the core lipid pool of soils, the relative abundance of brGDGTs (mean 71 ± 22%) was greater than that of the isoGDGTs (mean 22 ± 21%), whereas in the intact polar lipid fraction the archaeal isoGDGTs were comparable to the brGDGTs, having a mean relative concentration of 5 ± 5% vs 2 ± 2% (Supplementary Fig. S2). These values were lower than those detected in the Frasne peatland (Huguet et al., 2013, 2017) and in the Saxnäs Mosse (Peterse et al., 2011). However, they are comparable to results in soils close to the Sand Pond in the USA (Tierney et al., 2012) and a peat bog in northern Germany (Liu et al., 2010). In soils, faster production and degradation rates of bacteria-derived brGDGTs (lower standing stocks of living bacteria) relative to archaea-derived isoGDGTs were evidenced by the lower abundance of intact polar derived brGDGTs vs isoGDGTs, and inversely, the higher abundance of core brGDGTs relative to their isoprenoid analogues (Supplementary Fig. S2). Such an opposite relationship was previously found by Liu et al. (2010) in peat bog samples from the Bullenmoor (northern Germany), although these authors examined GDGTs in their intact form instead of the acid-hydrolyzed polar lipid fraction. Similarly, they suggested that the reverse pattern may be a result of higher activity and regeneration of microbes producing brGDGTs rather than by the producers of isoGDGTs in soils. Using SIP experiments, Huguet et al. (2017) found that the production rate of brGDGTs in the intact polar lipid pools was approximately 20 times slower than the core lipids in bog and fen acrotelm. This suggested low production and a high rate of degradation of intact polar brGDGTs, or at least lower standing stocks of living, relative to dead, bacteria.

Table 2
Concentrations (ng/g TOC) for individual GDGTs in the core and intact polar lipid fractions.

No.	iso GDGT-0	iso GDGT-1	iso GDGT-2	iso GDGT-3	Crenarchaeol	Crenarchaeol'	$IIIa_5$	$IIIa_6$	$IIIb_5$	$IIIb_6$	IIa_5	IIa_6	IIb_5	IIb_6	IIc_5	IIc_6	Ia	Ib	Ic
Core lipid fraction - soils																			
1	285	40	45	25	390	23	935	98	n.d.	n.d.	3739	765	261	158	25	n.d.	2502	396	167
2	17	41	35	9	159	6	536	47	n.d.	n.d.	3449	619	61	n.d.	27	n.d.	3920	149	53
3	3	5	2	2	7	n.d.	552	36	16	n.d.	3057	414	56	21	12	n.d.	3048	129	33
4	82	61	51	29	432	27	581	39	57	39	3098	584	65	24	18	n.d.	3223	129	36
5	1573	67	77	35	752	40	2666	243	37	17	10,727	1989	790	237	42	n.d.	6879	1088	326
6	3963	200	181	91	1916	150	813	530	111	80	2552	1601	346	1043	36	28	1764	584	108
7	3474	89	102	44	608	34	415	61	15	4	2018	350	133	95	13	n.d.	1532	178	39
8	2	8	7	3	18	n.d.	705	66	n.d.	n.d.	3441	664	68	39	14	n.d.	2972	142	46
9	6	3	n.d.	2	6	n.d.	708	61	n.d.	n.d.	3651	457	77	40	12	n.d.	2887	138	94
10	5734	379	374	142	2754	162	257	570	164	81	223	599	504	1018	46	73	351	307	151
11	10,598	416	406	177	2973	260	286	570	134	89	568	1029	177	1025	41	32	717	297	126
X1	1354	238	209	105	1894	147	371	687	133	59	269	682	201	873	30	31	684	239	93
X2	288	172	182	55	1659	129	111	575	234	103	108	535	140	857	75	47	278	277	215
X3	288	158	167	82	1509	127	285	685	155	122	349	1089	269	1330	63	44	505	343	142
X4	3	3	4	3	10	n.d.	768	90	n.d.	n.d.	4107	612	103	137	22	n.d.	3163	218	156
X5	99	75	58	34	540	26	914	562	78	53	2470	1847	823	1002	91	29	1497	885	597
X6	6	20	17	10	133	8	640	300	31	17	2494	985	313	385	41	11	1860	402	246
X7	34	73	63	38	563	35	598	617	64	14	1529	1469	421	762	94	15	1102	481	327
X8	91	124	100	55	776	51	623	686	86	19	1854	1451	421	1060	113	23	1360	461	302
H3	3096	539	458	249	3823	241	418	842	208	97	1613	931	206	1337	60	44	2495	458	156
H4	110	105	100	47	723	55	202	332	74	56	258	470	134	667	27	25	329	266	69
H5	379	195	183	103	1248	113	282	413	88	59	196	425	53	675	20	20	784	196	74
Core lipid fraction - groundwater SPMs																			
H32	46	35	29	7	107	4	78	30	18	8	145	50	36	18	5	2	206	23	7
H41	124	43	17	3	160	3	139	43	18	8	39	19	20	19	2	2	7	4	1
H42	14	8	5	0	7	0	376	18	29	1	66	20	26	7	3	n.d.	10	5	1
H43	13	5	1	1	6	0	753	53	145	7	127	44	101	8	11	0	18	9	3
H51	54	15	5	2	66	1	27	5	6	1	8	7	14	3	1	0	2	2	0
H52	14	6	3	1	15	0	99	78	3	49	33	28	4	72	2	8	8	5	1
H53	161	69	30	7	271	6	34	139	3	23	58	128	16	96	5	4	50	18	9
Intact polar lipid fraction - soils																			
1	74	40	46	21	182	19	41	3	n.d.	n.d.	186	44	12	8	n.d.	n.d.	130	14	7
2	8	15	11	3	17	1	17	1	n.d.	n.d.	208	101	14	n.d.	n.d.	n.d.	174	10	2
3	4	6	1	n.d.	2	n.d.	28	1	n.d.	n.d.	219	85	n.d.	n.d.	n.d.	n.d.	205	7	n.d.
4	46	192	200	79	1903	106	6	16	11	2	8	23	4	53	1	2	14	11	5
5	196	21	19	13	515	24	18	2	n.d.	n.d.	90	17	6	3	n.d.	n.d.	53	7	2
6	132	30	34	12	131	10	10	7	n.d.	n.d.	46	23	5	20	n.d.	n.d.	34	9	1
7	294	66	77	28	297	27	14	1	n.d.	n.d.	96	20	5	5	n.d.	n.d.	64	6	1
8	1	4	1	n.d.	2	n.d.	23	2	n.d.	n.d.	188	50	n.d.	n.d.	n.d.	n.d.	147	7	1
9	1	1	n.d.	n.d.	1	n.d.	14	1	n.d.	n.d.	115	35	n.d.	n.d.	n.d.	n.d.	88	6	1
10	14	18	14	7	78	5	8	4	n.d.	n.d.	24	10	2	7	n.d.	n.d.	16	7	5
11	449	140	144	59	1240	112	4	9	2	1	10	13	2	16	n.d.	n.d.	10	4	1
X1	266	43	42	17	600	41	5	10	3	1	6	12	3	21	n.d.	n.d.	15	5	2
X2	33	46	52	16	555	44	1	7	n.d.	n.d.	2	8	2	20	1	1	4	4	3
X3	40	41	47	23	372	42	9	25	n.d.	n.d.	16	46	10	61	2	2	24	18	6
X4	1	1	n.d.	n.d.	4	n.d.	32	4	n.d.	n.d.	240	45	n.d.	n.d.	n.d.	n.d.	167	13	7
X5	13	18	15	6	85	6	11	6	n.d.	n.d.	40	16	5	18	n.d.	n.d.	25	12	9
X6	1	4	4	1	26	2	7	3	2	n.d.	44	14	1	7	n.d.	n.d.	32	8	3
X7	2	17	15	8	141	8	17	17	1	2	60	44	8	31	3	n.d.	40	17	12
X8	3	19	17	8	147	10	10	10	n.d.	n.d.	42	18	1	19	2	n.d.	26	10	5
H3	458	67	53	38	1315	49	7	13	n.d.	n.d.	47	21	4	29	n.d.	n.d.	61	12	3
H4	3	12	10	6	203	10	4	8	3	1	8	13	3	22	0	1	10	8	2
H5	15	15	13	9	371	16	3	4	n.d.	n.d.	4	6	1	10	n.d.	n.d.	13	3	1
Intact polar lipid fraction – groundwater SPMs																			
H32	42	19	16	5	36	4	7	2	1	0	15	5	3	2	n.d.	n.d.	27	2	1
H41	64	23	17	6	59	2	35	8	3	2	14	5	4	5	1	0	4	1	0
H42	18	2	2	0	2	0	63	4	4	0	17	3	5	1	1	n.d.	2	1	0
H43	21	5	2	0	2	0	102	11	20	1	22	8	15	1	2	0	4	2	1
H51	20	8	6	2	23	1	4	1	1	0	1	1	2	0	n.d.	n.d.	0	0	n.d.
H52	16	9	5	1	7	0	31	21	1	12	14	9	1	23	1	2	3	2	0
H53	71	35	26	9	115	3	17	38	1	6	34	46	8	33	2	1	24	8	5

n.d. = not detected. The HTU and HTL states are for the Hainich transect upper and low aquifer, respectively.

Like in the soil samples, in groundwater SPM the core brGDGTs were more abundant than the core isoGDGTs (57 ± 5% vs 20 ± 3%), while the relative abundances of intact polar branched (12 ± 1%) and isoprenoid (11 ± 1%) GDGTs were comparable (Supplementary Fig. S2). Such trends might indicate that: (1) brGDGT-producing bacteria have been more abundant than isoGDGT-producing archaea over time, or (2) isoGDGT-producing archaea have very low rates of cell division while alive.

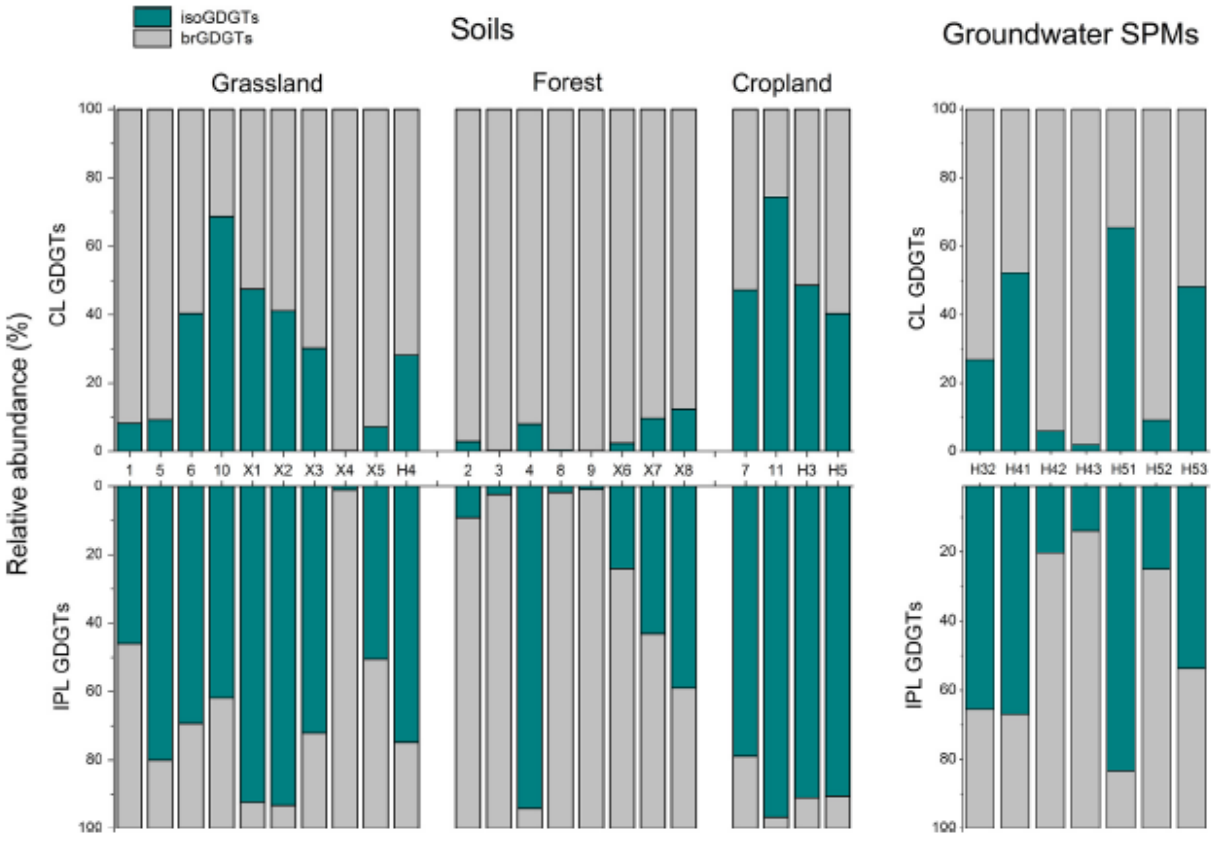


Fig. 4. Relative abundance of brGDGTs and isoGDGTs in the form of core and intact polar lipid fractions in surface soils (left) and aquifer SPM (right).

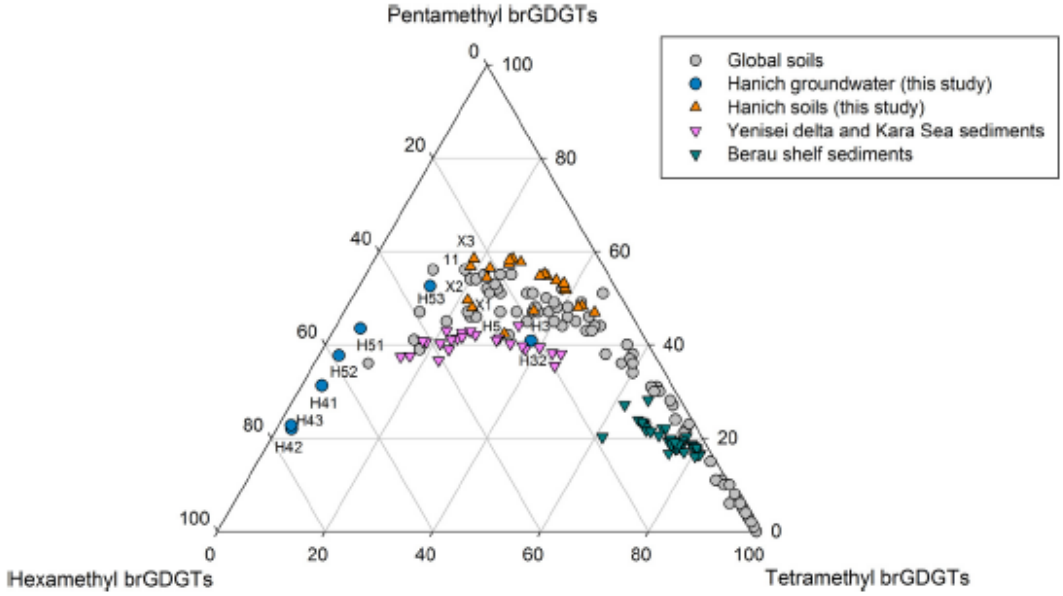


Fig. 5. Composition of core brGDGTs in soils, sediments, and groundwater SPM: The three end-members of the Ternary diagram represent the fractional abundance of tetra-, penta- and hexamethylated brGDGTs. Details can be found in Sinninghe Damsté (2016). Datasets for global soils are from De Jonge et al. (2014), from De Jonge et al. (2015b) for the Yenisei Delta and Kara Sea sediments, and from Sinninghe Damsté (2016) for the Berau Shelf sediments.

5.2. Source of GDGTs in the aquifers

5.2.1. Origin of bacteria-derived branched GDGTs

Tracing the origin of GDGT-producing microorganisms is fundamental for understanding how GDGTs function in environments. Previous studies have shown that terrestrial sources can significantly contribute to the brGDGT pool in riverine, coastal, and lacustrine environments (Loomis et al., 2014; Naeher et al., 2014; De Jonge et al., 2015a). Higher br- vs isoGDGTs (in both core and intact polar pools) in groundwater of the sites H42 and H43, and the proximity of those sites to the Eichbach River, might suggest inputs of brGDGT with river water infiltration (Fig. 2). However, those

aquifers are confined at the top and base by unfractured or poorly permeable marlstone layers, which means that groundwater recharge occurs mainly from sites located uphill of the transect (horizontal input: Kohlhepp et al., 2017). Therefore, major contribution of brGDGTs from the river into those wells is unlikely. As mentioned below, another explanation could be that brGDGTs are produced by in situ anaerobic bacteria (Weijers et al., 2006, 2011).

The sources of the brGDGTs have been additionally inferred from their degree of methylation (Sinninghe Damsté et al., 2016), as shown in Fig. 5. Our soil samples were dominated by either tetra- or penta-methylated brGDGTs. This is consistent with temperate climate soil development because tropical soils typically have a higher relative contribution of tetramethylated branched GDGTs (Weijers et al., 2007). This decreasing number of branched methyl groups in branched GDGTs is commonly interpreted as an adaption strategy of branched GDGT-producing bacteria to cope with increasing temperature (Weijers et al., 2007). That the microbial communities in the groundwater samples H32 and H53 differed from those in other groundwater SPM, is shown by greater contributions of tetra- or penta-methylated brGDGTs. Data from those two sites plotted closely to those of global soils and to the soils of their respective recharge areas (X1–X3, 11, H3, and H5), which likely indicates the transport of soil organic matter to the groundwater.

Thick soils and low-permeability cap rocks (see Fig. 2) above the aquifer HTU/moM-8 (or well H53) likely isolate the groundwater from vertical input. Here, recharge likely occurs horizontally (recharge area X1–X3, Fig. 1). The low permeability aquifer bedrock causes recharge waters to percolate slowly, which allows for complete O_2 consumption, low redox potential, and chemical equilibration with components of the bedrock in the groundwater of well H53 (Küsel et al., 2016; Kohlhepp et al., 2017, Nowak et al., 2017). Inversely, the sinkholes in the shoulder and midslope of the CZE-transect more effectively connect the forest and grassland soils (recharge areas 1–3, 5, 7–9) to the aquifer HTU/moM-4 (or well H32, Fig. 2). Thus, this particular setting supports the notion that those soils are a potential source of the tetra- or penta-methylated brGDGT in well H32. Here, faster transport to the subsurface favors sub-oxic condition of the groundwater and may benefit surface organisms surviving within such "hostile" environments (Goldscheider et al., 2006; Schwab et al., 2017).

In contrast to these two sites, the composition of brGDGTs in the groundwater of wells H41–H43, H51, and H52 clearly deviate from that of the surface soil of their recharge areas, suggesting very low or no terrestrial input into the groundwater. The particularly high predominance of hexamethylated brGDGTs (on average 65% of the total brGDGTs, n = 5) in the ternary diagram of brGDGTs (Fig. 5) shows an uncommon brGDGT composition and in situ production by some unknown bacteria (Sinninghe Damsté et al., 2016). Considering the strong difference in the patterns of composition and concentration of brGDGT between groundwater and soils, it would be expected that even a small input of terrestrially derived brGDGTs would significantly alter the distribution pattern of brGDGTs in groundwater. A small temperature variation in the groundwater (9.7–10.6 °C, Table 1) indicates that temperature changes are not the main drivers of the degree of methylation of the brGDGTs in the groundwater.

Ingalls et al. (2012) and French et al. (2015) used the correlation between concentrations of core and intact polar GDGTs to determine their origin in river and marine environments. In the studied samples, the core and intact polar brGDGTs were not correlated in the soils (Fig. 6a), but were significantly correlated in the groundwater ($R^2 = 0.80$, n = 5, p = 0.02, Fig. 6c), after removing the two possible outliers (H32 and H53; Fig. 5). This trend further supported terrestrial brGDGT inputs in H32 and H53, and also indicated low disturbance (e.g., little surface input) in the other groundwater samples that had indigenous bacterial communities.

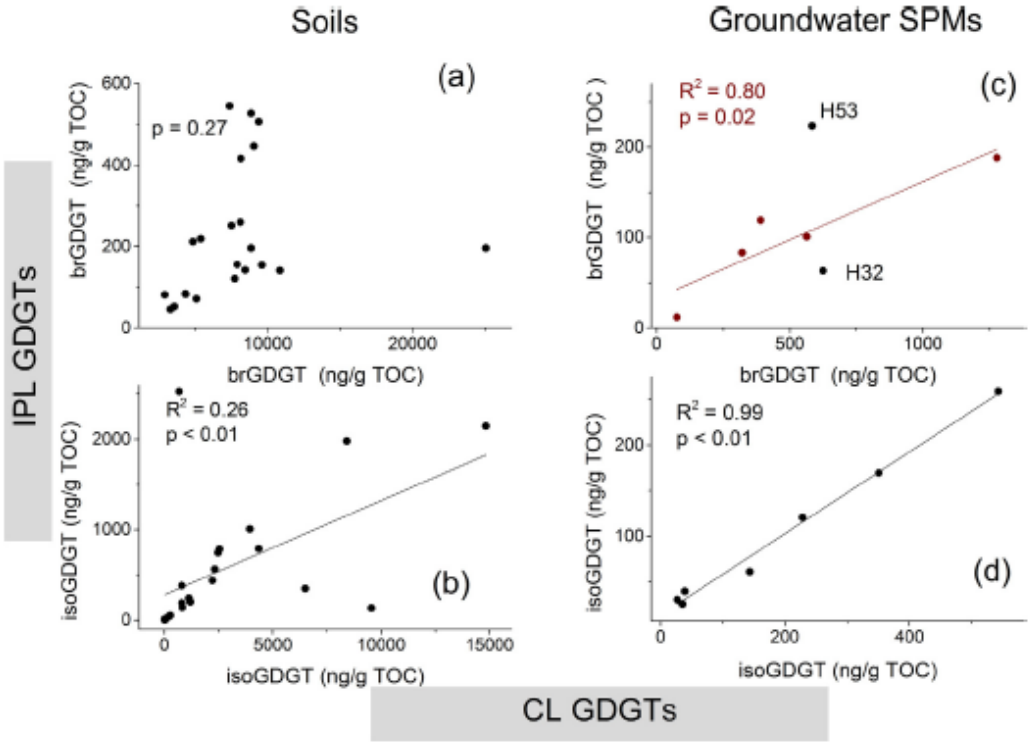


Fig. 6. Linear regression plot of core and intact polar GDGTs in soils (left) and groundwater SPM (right). The upper ones show brGDGTs in soils (a) and in groundwater SPM (c). The lower ones show isoGDGTs in soils (b) and in groundwater SPM (d). Different axes are used.

Table 3
Correlation of archaeal intact polar GDGT concentrations with four dominant phylotypes in the aquifers.

		MG-I	SITS412	M + S
isoGDGTs	Correlation coefficient r	0.85*	−0.41	0.85*
	p value	0.02	0.36	0.02

Note: The capital letters M and S stand for archaeal community MG-I and SITS412, respectively.
Dominant phylotypes were described by Lazar et al. (2017).
MG-I: Marine Group I (MG-I or group 1.1a) Thaumarchaeota.
SITS412: the SITS412 Thermoplasmatales.
* Correlation is significant at the 0.05 level (2-tailed).

5.2.2. Origin of archaea-derived isoprenoid GDGTs

As shown in Section 5.2.1, a poor correlation was also found between core and intact polar isoGDGTs in soils ($R^2 = 0.26$, $p < 0.01$, $n = 22$, Fig. 6b). In contrast, in groundwater, a strong correlation was found between core and intact polar isoGDGTs ($R^2 = 0.99$, $p < 0.01$, $n = 7$, Fig. 6d). This trend has been previously observed by French et al. (2015) in estuarine and marine environments and similarly may suggest that the production and preservation of isoGDGTs in aquifers are well coupled with little disturbances from allochthonous sources.

Further evidence of the producers of isoGDGTs in the groundwater was provided by comparing the relative abundance of isoGDGTs with sequences of archaeal 16S rRNA genes (Lazar et al., 2017). Although this approach may suffer from significant biases (DNA and lipids may have different residence and transport times), it might still help to elucidate how the lipid compositions and the microbial communities link in distinct environments (e.g., Pitcher et al., 2011, Schouten et al., 2012). Such a comparison may be particularly relevant here, because as discussed above, the groundwater environments displayed little disturbance (e.g., not much surface input).

According to a study by Lazar et al. (2017), the archaeal communities found in the soil samples of the Hainich CZE were mainly dominated by the Crenarchaeotal Group 1.1c and Soil Crenarchaeota Group (SCG) 1.1b. However, in the studied aquifers, Marine Group I (MG-I or group 1.1a) Thaumarchaeota, as well as Rice Cluster V (RC-V) and the Deep-sea Hydrothermal Vent Euryarchaeota group 6 (DHVE-6) Woesearchaeota, were the most abundant DNA-based archaeal communities (Lazar et al., 2017). MG-I Thaumarchaeota are known to be a major source of isoGDGTs (Schouten et al., 2013; Elling et al., 2017), although the SITS412 Thermoplasmatales have been shown to be additional producers of isoGDGTs 0–4 (Macalady et al., 2004).

To determine in which proportion the different isoGDGT-producers may have contributed to the isoGDGT distributions in the groundwater, we compared the relative concentrations of total intact polar isoGDGTs with the relative abundance of the related phylotypes described by Lazar et al. (2017). The high correlation between the MG-I and the total isoGDGTs ($r = 0.85$, $p = 0.02$, Table 3) indicated that MG-I Thaumarchaeota were major isoGDGT-producers in groundwater. This, and the fact that ca. 0.03% of the SCG contribute to the groundwater archaea in the upper aquifers (Lazar et al., 2017), further supported the notion that the subsurface isoGDGTs were mainly produced in situ, most likely by aerobic ammonia-oxidizing archaea (Group 1.1a Thaumarchaeota; Schouten et al., 2012). The observed correlation between the summed MG-I and SITS412 (M + S) and the total isoGDGTs (Table 3) may reflect the strong correlation between MG-I and the total isoGDGTs. This is particularly likely because SITS412 are in relatively low abundance, and thus, the notion that SITS412 is an isoGDGT-producer in that groundwater is not clearly supported.

6. Conclusions

This first report of tetraether lipids in groundwater shows a distribution typical of brGDGTs, as characterized by a strong predominance of hexamethylated tetraether lipids. An opposite relationship of archaea-derived isoGDGTs vs bacteria-derived brGDGTs in intact polar lipids vs core lipids was found in topsoils, confirming that in such environments the bacterial producers of GDGTs are more active and have faster regeneration rates than archaea producers. In groundwater, the similar proportion of intact polar lipids GDGTs suggested that the activity of brGDGT-producing bacteria was higher than that of isoGDGT-producing archaea. The composition of brGDGTs indicated that soil inputs from the respective recharge areas likely influenced two groundwater samples (named H32 and H53). BrGDGTs in other wells were mainly produced in situ, by some bacteria unique to the groundwater environments. The strong correlation between core and intact polar isoGDGTs in the groundwater suggested little disturbances from allochthonous source inputs and likely the presence of indigenous GDGT-producing archaea communities. The 16S rRNA archaeal gene data supported the in situ production of archaea-derived isoGDGTs in aquifers; mainly from Group 1.1a Thaumarchaeota. This study indicates that GDGTs can be useful biomarkers for tracing soil microbial inputs to groundwater.

Acknowledgements

We would like to thank Robert Lehmann for organizing field trips and sampling of groundwater; Ines Hike, Heiko Moossen, and Iris Kuhlmann for measuring the element parameters; and Somak Chowdhury and Xiu Jia for discussing the 16S rRNA gene data. Markus Lange, Vanessa-Nina Roth, Yunping Xu, and Cassandre S. Lazar are thanked for providing helpful comments on an earlier version of this paper. Jessica E. Tierney suggested the hydrolysis methods used in this study. We would like to thank the two anonymous reviewers for their positive and insightful comments on the manuscript. This study is part of the Collaborative Research Centre AquaDiva (CRC 1076 AquaDiva) of the Friedrich Schiller Universität Jena, funded by the Deutsche Forschungsgemeinschaft. The IMPRS-gBGC provided the funding for the PhD scholarship of Su Ding.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.orggeochem.2018.10.005.

Associate Editor—**Ann Pearson**

References

Akob, D.M., Küsel, K., 2011. Where microorganisms meet rocks in the Earth's Critical Zone. Biogeosciences 8, 3531–3543.

Anderson, V.J., Shanahan, T.M., Saylor, J.E., Horton, B.K., Mora, A.R., 2014. Sources of local and regional variability in the MBT'/CBT paleotemperature proxy: insights from a modern elevation transect across the Eastern Cordillera of Colombia. Organic Geochemistry 69, 42–51.

Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T.J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H., Hinrichs, K.-U., 2006. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. Proceedings of the National Academy of Sciences of the United States of America 103, 3846–3851.

Blyth, A.J., Jex, C.N., Baker, A., Khan, S.J., Schouten, S., 2014. Contrasting distributions of glycerol dialkyl glycerol tetraethers (GDGTs) in speleothems and associated soils. Organic Geochemistry 69, 1–10.

Buckles, L.K., Weijers, J.W.H., Verschuren, D., Sinninghe Damsté, J.S., 2014. Sources of core and intact branched tetraether membrane lipids in the lacustrine environment: anatomy of Lake Challa and its catchment, equatorial East Africa. Geochimica et Cosmochimica Acta 140, 106–126.

Danielopol, D.L., Griebler, C., Gunatilaka, A., Notenboom, J., 2003. Present state and future prospects for groundwater ecosystems. Environmental Conservation 30, 104–130.

De Jonge, C., Hopmans, E.C., Zell, C.I., Kim, J.-H., Schouten, S., Sinninghe Damsté, J.S., 2014. Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: implications for palaeoclimate reconstruction. Geochimica et Cosmochimica Acta 141, 97–112.

De Jonge, C., Stadnitskaia, A., Fedotov, A., Sinninghe Damsté, J.S., 2015a. Impact of riverine suspended particulate matter on the branched glycerol dialkyl glycerol tetraether composition of lakes: the outflow of the Selenga River in Lake Baikal (Russia). Organic Geochemistry 83–84, 241–252.

De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Streletskaya, I.D., Vasiliev, A.A., Sinninghe Damsté, J.S., 2015b. Drastic changes in the distribution of branched tetraether lipids in suspended matter and sediments from the Yenisei River and Kara Sea (Siberia): implications for the use of brGDGT-based proxies in coastal marine sediments. Geochimica et Cosmochimica Acta 165, 200–225.

Detmers, J., Strauss, H., Schulte, U., Bergmann, A., Knittel, K., Kuever, J., 2004. FISH shows that *Desulfotomaculum* spp. are the dominating sulfate-reducing bacteria in a pristine aquifer. Microbial Ecology 47, 236–242.

Ding, S., Xu, Y., Wang, Y., He, Y., Hou, J., Chen, L., He, J.S., 2015. Distribution of branched glycerol dialkyl glycerol tetraethers in surface soils of the Qinghai-Tibetan Plateau: implications of brGDGTs-based proxies in cold and dry regions. Biogeosciences 12, 3141–3151.

Ding, S., Schwab, V.F., Ueberschaar, N., Roth, V.-N., Lange, M., Xu, Y., Gleixner, G., Pohnert, G., 2016. Identification of novel 7-methyl and cyclopentanyl branched glycerol dialkyl glycerol tetraethers in lake sediments. Organic Geochemistry 102, 52–58.

Elling, F.J., Könneke, M., Nicol, G.W., Stieglmeier, M., Bayer, B., Spieck, E., de la Torre, J.R., Becker, K.W., Thomm, M., Prosser, J.I., Herndl, G.J., Schleper, C., Hinrichs, K.-U., 2017. Chemotaxonomic characterisation of the thaumarchaeal lipidome. Environmental Microbiology 19, 2681–2700.

French, D.W., Huguet, C., Turich, C., Wakeham, S.G., Carlson, L.T., Ingalls, A.E., 2015. Spatial distributions of core and intact glycerol dialkyl glycerol tetraethers (GDGTs) in the Columbia River Basin and Willapa Bay, Washington: insights into origin and implications for the BIT index. Organic Geochemistry 88, 91–112.

Günther, F., Thiele, A., Gleixner, G., Xu, B., Yao, T., Schouten, S., 2014. Distribution of bacterial and archaeal ether lipids in soils and surface sediments of Tibetan lakes: implications for GDGT-based proxies in saline high mountain lakes. Organic Geochemistry 67, 19–30.

Goldscheider, N., Hunkeler, D., Rossi, P., 2006. Review: Microbial biocenoses in pristine aquifers and an assessment of investigative methods. Hydrogeology Journal 14, 926–941.

Green, C.T., Scow, K.M., 2000. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeology Journal 8, 126–141.

Griebler, C., Lueders, T., 2009. Microbial biodiversity in groundwater ecosystems. Freshwater Biology 54, 649–677.

Harvey, H.R., Fallon, R.D., Patton, J.S., 1986. The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. Geochimica et Cosmochimica Acta 50, 795–804.

Herrmann, M., Rusznyák, A., Akob, D.M., Schulze, I., Opitz, S., Totsche, K.U., Küsel, K., 2015. Large fractions of CO_2-fixing microorganisms in pristine limestone aquifers appear to be involved in the oxidation of reduced sulfur and nitrogen compounds. Applied and Environmental Microbiology 81, 2384–2394.

Herrmann, M., Opitz, S., Harzer, R., Totsche, K., Küsel, K., 2017. Attached and suspended denitrifier communities in pristine limestone aquifers harbor high fractions of potential autotrophs oxidizing reduced iron and sulfur compounds. Microbial Ecology 74, 264–277.

Huguet, A., Fosse, C., Laggoun-Défarge, F., Delarue, F., Derenne, S., 2013. Effects of a short-term experimental microclimate warming on the abundance and distribution of branched GDGTs in a French peatland. Geochimica et Cosmochimica Acta 105, 294–315.

Huguet, A., Meador, T.B., Laggoun-Défarge, F., Könneke, M., Wu, W., Derenne, S., Hinrichs, K.-U., 2017. Production rates of bacterial tetraether lipids and fatty acids in peatland under varying oxygen concentrations. Geochimica et Cosmochimica Acta 203, 103–116.

Huguet, C., Martens-Habbena, W., Urakawa, H., Stahl, D.A., Ingalls, A.E., 2010. Comparison of extraction methods for quantitative analysis of core and intact polar glycerol dialkyl glycerol tetraethers (GDGTs) in environmental samples. Limnology and Oceanography: Methods 8, 127–145.

Huguet, C., Hopmans, E.C., Febo-Ayala, W., Thompson, D.H., Sinninghe Damsté, J.S., Schouten, S., 2006. An improved method to determine the absolute abundance of glycerol dibiphytanyl glycerol tetraether lipids. Organic Geochemistry 37, 1036–1041.

Ingalls, A.E., Huguet, C., Truxal, L.T., 2012. Distribution of intact and core membrane lipids of archaeal glycerol dialkyl glycerol tetraethers among size-fractionated particulate organic matter in Hood Canal, Puget Sound. Applied and Environmental Microbiology 78, 1480–1490.

Jørgensen, B.B., Marshall, I.P.G., 2016. Slow microbial life in the seabed. Annual Review of Marine Science 8, 311–332.

Kates, M., 1993. Membrane lipids of Archaea. In: Kates, M., Kushner, D.J., Matheson, A.T. (Eds.), The Biochemistry of Archaea (Archaebacteria). Elsevier Biomedical Press, Amsterdam, The Netherlands, pp. 261–295.

Könneke, M., Bernhard, A.E., José, R., Walker, C.B., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546.

Koga, Y., Morii, H., 2005. Recent advances in structural research on ether lipids from archaea including comparative and physiological aspects. Bioscience, Biotechnology and Biochemistry 69, 2019–2034.

Kohlhepp, B., Lehmann, R., Seeber, P., Küsel, K., Trumbore, S.E., Totsche, K.U., 2017. Aquifer configuration and geostructural links control the groundwater quality in thin-bedded carbonate–siliciclastic alternations of the Hainich CZE, central Germany. Hydrology and Earth System Sciences 21, 6091–6116.

Küsel, K., Totsche, K.U., Trumbore, S.E., Lehmann, R., Steinhäuser, C., Herrmann, M., 2016. How deep can surface signals be traced in the critical zone? Merging biodiversity with biogeochemistry research in a central German Muschelkalk landscape. Frontiers in Earth Science 4, 32. https://doi.org/10.3389/feart.2016.00032.

Lazar, C.S., Stoll, W., Lehmann, R., Herrmann, M., Schwab, V.F., Akob, D.M., Nawaz, A., Wubet, T., Buscot, F., Totsche, K.-U., Küsel, K., 2017. Archaeal diversity and CO2 fixers in carbonate-/siliciclastic-rock groundwater ecosystems. Archaea paper 2136287. https://doi.org/10.1155/2017/2136287.

Lipp, J.S., Morono, Y., Inagaki, F., Hinrichs, K.-U., 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. Nature 454, 991–994.

Lipp, J.S., Hinrichs, K.-U., 2009. Structural diversity and fate of intact polar lipids in marine sediments. Geochimica et Cosmochimica Acta 73, 6816–6833.

Liu, X., Leider, A., Gillespie, A., Gröger, J., Versteegh, G.J.M., Hinrichs, K.-U., 2010. Identification of polar lipid precursors of the ubiquitous branched GDGT orphan lipids in a peat bog in Northern Germany. Organic Geochemistry 41, 653–660.

Liu, X., Lipp, J.S., Hinrichs, K.-U., 2011. Distribution of intact and core GDGTs in marine sediments. Organic Geochemistry 42, 368–375.

Logemann, J., Graue, J., Köster, J., Engelen, B., Rullkötter, J., Cypionka, H., 2011. A laboratory experiment of intact polar lipid degradation in sandy sediments. Biogeosciences 8, 2547–2560.

Loomis, S.E., Russell, J.M., Heureux, A.M., D'Andrea, W.J., Sinninghe Damsté, J.S., 2014. Seasonal variability of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in a temperate lake system. Geochimica et Cosmochimica Acta 144, 173–187.

Martino, D.P., Grossman, E.L., Ulrich, G.A., Burger, K.C., Schlichenmeyer, J.L., Suflita, J. M., Ammerman, J.W., 1998. Microbial abundance and activity in a low-conductivity aquifer system in East-Central Texas. Microbial Ecology 35, 224–234.

Macalady, J.L., Vestling, M.M., Baumler, D., Boekelheide, N., Kaspar, C.W., Banfield, J. F., 2004. Tetraether-linked membrane monolayers in *Ferroplasma* spp: a key to survival in acid. Extremophiles 8, 411–419.

Naeher, S., Peterse, F., Smittenberg, R.H., Niemann, H., Zigah, P.K., Schubert, C.J., 2014. Sources of glycerol dialkyl glycerol tetraethers (GDGTs) in catchment soils, water column and sediments of Lake Rotsee (Switzerland) – implications for the application of GDGT-based proxies for lakes. Organic Geochemistry 66, 164–173.

Nambiar, E.S., Ranger, J., Tiarks, A., Toma, T., 2004. Site management and productivity in tropical plantation forests: proceedings of workshops in Congo, July 2001 and China, February 2003. Center for International Forestry Research.

Nawaz, A., Purahong, W., Lehmann, R., Herrmann, M., Küsel, K., Totsche, K.U., Buscot, F., Wubet, T., 2016. Superimposed pristine limestone aquifers with marked hydrochemical differences exhibit distinct fungal communities. Frontiers in Microbiology 7, 666. https://doi.org/10.3389/fmicb.2016.00666.

Nowak, M.E., Schwab, V.F., Lazar, C.S., Behrendt, T., Kohlhepp, B., Totsche, K.U., Küsel, K., Trumbore, S.E., 2017. Carbon isotopes of dissolved inorganic carbon reflect utilization of different carbon sources by microbial communities in two limestone aquifer assemblages. Hydrology and Earth System Sciences 21, 4283–4300.

Oba, M., Sakata, S., Tsunogai, U., 2006. Polar and neutral isopranyl glycerol ether lipids as biomarkers of archaea in near-surface sediments from the Nankai Trough. Organic Geochemistry 37, 1643–1654.

Opitz, S., Küsel, K., Spott, O., Totsche, K.U., Herrmann, M., 2014. Oxygen availability and distance to surface environments determine community composition and abundance of ammonia-oxidizing prokaroytes in two superimposed pristine limestone aquifers in the Hainich region, Germany. FEMS Microbiology Ecology 90, 39–53.

Peterse, F., Hopmans, E.C., Schouten, S., Mets, A., Rijpstra, W.I.C., Sinninghe Damsté, J.S., 2011. Identification and distribution of intact polar branched tetraether lipids in peat and soil. Organic Geochemistry 42, 1007–1015.

Pitcher, A., Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2009. Separation of core and intact polar archaeal tetraether lipids using silica columns: Insights into living and fossil biomass contributions. Organic Geochemistry 40, 12–19.

Pitcher, A., Rychlik, N., Hopmans, E.C., Spieck, E., Rijpstra, W.I.C., Ossebaar, J., Schouten, S., Wagner, M., Sinninghe Damsté, J.S., 2010. Crenarchaeol dominates the membrane lipids of *Candidatus* Nitrososphaera gargensis, a thermophilic Group I.1b Archaeon. The ISME Journal 4, 542–552.

Pitcher, A., Hopmans, E.C., Mosier, A.C., Park, S.-J., Rhee, S.-K., Francis, C.A., Schouten, S., Sinninghe Damsté, J.S., 2011. Core and intact polar glycerol dibiphytanyl glycerol tetraether lipids of ammonia-oxidizing archaea enriched from marine and estuarine sediments. Applied and Environmental Microbiology 77, 3468–3477.

Risse-Buhl, U., Herrmann, M., Lange, P., Akob, D.M., Pizani, N., Schönborn, W., Totsche, K.U., Küsel, K., 2013. Phagotrophic protist diversity in the groundwater of a karstified aquifer – morphological and molecular analysis. Journal of Eukaryotic Microbiology 60, 467–479.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.

Schouten, S., Hopmans, E.C., Schefuß, E., Sinninghe Damsté, J.S., 2002. Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures? Earth and Planetary Science Letters 204, 265–274.

Schouten, S., Middelburg, J.J., Hopmans, E.C., Sinninghe Damsté, J.S., 2010. Fossilization and degradation of intact polar lipids in deep subsurface sediments: a theoretical approach. Geochimica et Cosmochimica Acta 74, 3806–3814.

Schouten, S., Pitcher, A., Hopmans, E.C., Villanueva, L., van Bleijswijk, J., Sinninghe Damsté, J.S., 2012. Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone: I. Selective preservation and degradation in the water column and consequences for the TEX_{86}. Geochimica et Cosmochimica Acta 98, 228–243.

Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. Organic Geochemistry 54, 19–61.

Schwab, V.F., Herrmann, M., Roth, V.N., Gleixner, G., Lehmann, R., Pohnert, G., Trumbore, S., Küsel, K., Totsche, K.U., 2017. Functional diversity of microbial communities in pristine aquifers inferred by PLFA- and sequencing-based approaches. Biogeosciences 14, 2697–2714.

Sinninghe Damsté, J.S., Schouten, S., Hopmans, E.C., van Duin, A.C.T., Geenevasen, J. A.J., 2002. Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. Journal of Lipid Research 43, 1641–1651.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Weijers, J.W.H., Foesel, B.U., Overmann, J., Dedysh, S.N., 2011. 13,16-Dimethyl octacosanedioic acid (iso-diabolic acid), a common membrane-spanning lipid of Acidobacteria subdivisions 1 and 3. Applied and Environmental Microbiology 77, 4147–4154.

Sinninghe Damsté, J.S., 2016. Spatial heterogeneity of sources of branched tetraethers in shelf systems: the geochemistry of tetraethers in the Berau River delta (Kalimantan, Indonesia). Geochimica et Cosmochimica Acta 186, 13–31.

Simkus, D.N., Slater, G.F., Lollar, B.S., Wilkie, K., Kieft, T.L., Magnabosco, C., Lau, M.C.Y., Pullin, M.J., Hendrickson, S.B., Wommack, K.E., Sakowski, E.G., Heerden, E.v., Kuloyo, O., Linage, B., Borgonie, G., Onstott, T.C., 2016. Variations in microbial carbon sources and cycling in the deep continental subsurface. Geochimica et Cosmochimica Acta 173, 264–283.

Starke, R., Müller, M., Gaspar, M., Marz, M., Küsel, K., Totsche, K.U., von Bergen, M., Jehmlich, N., 2017. Candidate Brocadiales dominates C, N and S cycling in anoxic groundwater of a pristine limestone-fracture aquifer. Journal of Proteomics 152, 153–160.

Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology. Rapid Communications in Mass Spectrometry 18, 617–628.

Teske, A., Sorensen, K.B., 2008. Uncultured archaea in deep marine subsurface sediments: have we caught them all? The ISME Journal 2, 3–18.

Tierney, J.E., Russell, J.M., 2009. Distributions of branched GDGTs in a tropical lake system: Implications for lacustrine application of the MBT/CBT paleoproxy. Organic Geochemistry 40, 1032–1036.

Tierney, J.E., Schouten, S., Pitcher, A., Hopmans, E.C., Sinninghe Damsté, J.S., 2012. Core and intact polar glycerol dialkyl glycerol tetraethers (GDGTs) in Sand Pond, Warwick, Rhode Island (USA): insights into the origin of lacustrine GDGTs. Geochimica et Cosmochimica Acta 77, 561–581.

Verardo, D.J., Froelich, P.N., McIntyre, A., 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer. Deep Sea Research Part A Oceanographic Research Papers 37, 157–165.

Weijers, J.W.H., Schouten, S., Hopmans, E.C., Geenevasen, J.A.J., David, O.R.P., Coleman, J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. Environmental Microbiology 8, 648–657.

Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007. Environmental controls on bacterial tetraether membrane lipid distribution in soils. Geochimica et Cosmochimica Acta 71, 703–713.

Weijers, J.W.H., Panoto, E., van Bleijswijk, J., Schouten, S., Rijpstra, W.I.C., Balk, M., Stams, A.J., Sinninghe Damsté, J.S., 2009. Constraints on the biological source(s) of the orphan branched tetraether membrane lipids. Geomicrobiology Journal 26, 402–414.

Weijers, J.W.H., Steinmann, P., Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2011. Bacterial tetraether membrane lipids in peat and coal: Testing the MBT–CBT temperature proxy for climate reconstruction. Organic Geochemistry 42, 477–486.

Wu, W., Ruan, J., Ding, S., Zhao, L., Xu, Y., Yang, H., Ding, W., Pei, Y., 2014. Source and distribution of glycerol dialkyl glycerol tetraethers along lower Yellow River-estuary–coast transect. Marine chemistry 158, 17–26.

Xie, S., Lipp, J.S., Wegener, G., Ferdelman, T.G., Hinrichs, K.-U., 2013. Turnover of microbial lipids in the deep biosphere and growth of benthic archaeal populations. Proceedings of the National Academy of Sciences 110, 6010–6014.

Yang, H., Lü, X., Ding, W., Lei, Y., Dang, X., Xie, S., 2015. The 6-methyl branched tetraethers significantly affect the performance of the methylation index (MBT′) in soils from an altitudinal transect at Mount Shennongjia. Organic Geochemistry 82, 42–53.

Zell, C., Kim, J.-H., Hollander, D., Lorenzoni, L., Baker, P., Silva, C.G., Nittrouer, C., Sinninghe Damsté, J.S., 2014. Sources and distributions of branched and isoprenoid tetraether lipids on the Amazon shelf and fan: Implications for the use of GDGT-based proxies in marine sediments. Geochimica et Cosmochimica Acta 139, 293–312.

Zinger, L., Gobet, A., Pommier, T., 2012. Two decades of describing the unseen majority of aquatic microbial diversity. Molecular Ecology 21, 1878–1896.

Supplementary Material

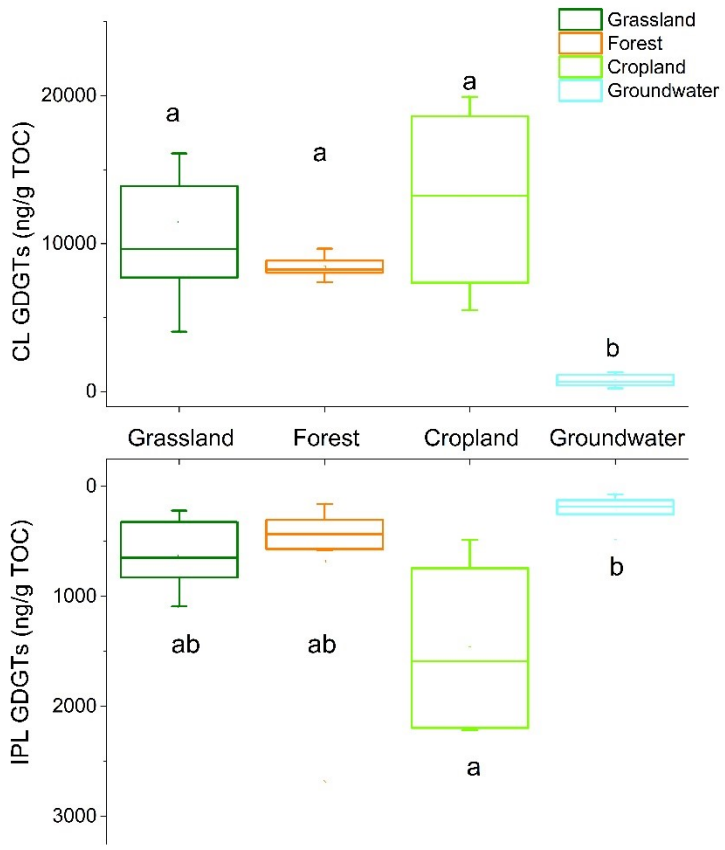


Fig. S1. Box plots showing the summed absolute abundances of core and intact polar GDGTs in both surface soils and pristine aquifer SPMs. Soils were separated into three groups by different vegetation types (grassland, forest and cropland). Different letters above bars denote samples that were significantly different ($P < 0.05$).

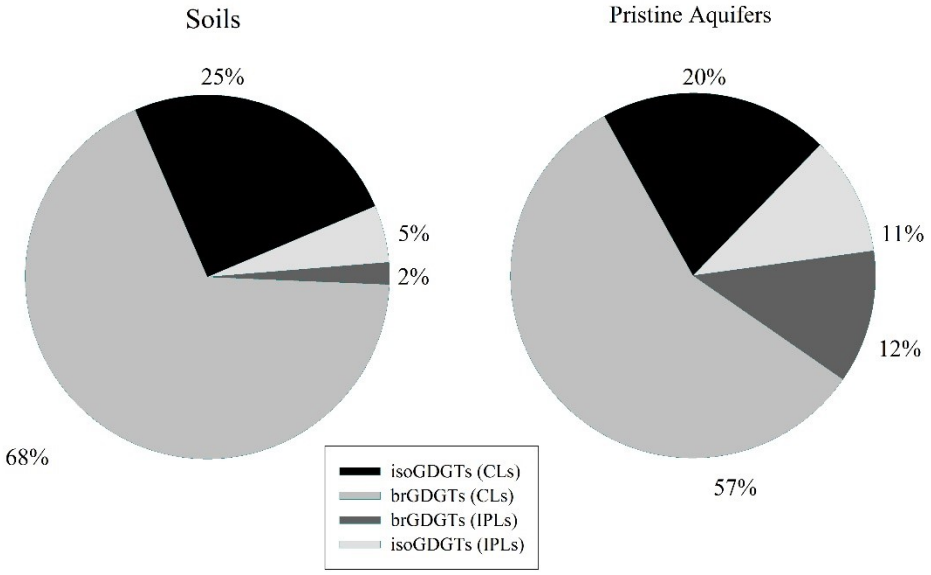


Fig. S2. Pie charts showing relative abundances of all core and intact polar GDGTs in soils (left) and aquifers (right).

Part II. Full lipid profile and their affect environmental parameters in soils

4. Characterization of intact polar lipids in soils for assessing their origin

Su Ding, Markus Lange, Julius Lipp, Valérie F. Schwab, Somak Chowdhury, Melanie Maraun, Katrin Krause, Dapeng Li, Erika Kothe, Stefan Scheu, Ruth Welti, Kai-Uwe Hinrichs, Gerd Gleixner

Submitted to *Soil Biology and Biochemistry*

Manuscript III

Statement on individual contributions of doctoral candidate in a cumulative doctoral thesis

Publication (complete citation):
Ding, S.[1], Lange, M.[2], Lipp, J.[3], Chowdhury, S.[4], Schwab, V.F.[5], Maraun, M.[6], Krause, K.[7], Kothe, E.[8], Li, D.[9], Scheu, S.[10], Welti, R.[11], Hinrichs, K.U.[12], Gleixner, G.[13], 2018. Characterization of intact polar lipids in soils for assessing their origin. submitted to *Soil Biology and Biochemistry*

Involved in	Author number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Conception of research approach	X	X										X	X
Planning of research activities	X	X	X									X	X
Data collection	X	X	X	X		X	X		X				
Data analysis and interpretation	X	X	X		X			X		X	X	X	X
Writing a manuscript	X	X			X						X		X
Suggested publication equivalence value to be	1.0												

The subsequent manuscript has not been used in any other cumulative PhD thesis. Reprint permissions for this publication were obtained from the publisher and the coauthors.

Characterization of intact polar lipids in soils for assessing their origin

Su Ding[a], Markus Lange[a], Julius Lipp[b], Valérie F. Schwab[a], Somak Chowdhury[a], Melanie M. Pollierer[c], Katrin Krause[d], Dapeng Li[e], Erika Kothe[d], Stefan Scheu[c], Ruth Welti[f], Kai-Uwe Hinrichs[b], Gerd Gleixner[a,*]

[a]*Max Planck Institute for Biogeochemistry, Hans-Knöll-Str. 10, 07745 Jena, Germany*

[b]*Organic Geochemistry Group, MARUM Center for Marine Environmental Sciences and Department of Geosciences, University of Bremen, Leobener Straße, 28359 Bremen, Germany*

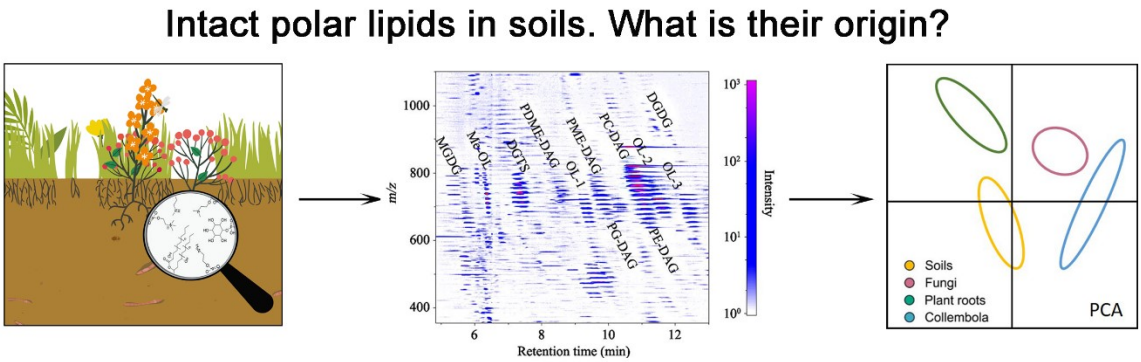
[c]*J.F. Blumenbach Institute of Zoology and Anthropology, Georg August University Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany*

[d]*Institute of Microbiology, Friedrich Schiller University of Jena, 07745 Jena, Germany*

[e]*Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany*

[f]*Division of Biology, Kansas State University, Manhattan, 66506 Kansas, USA*

Graphical abstract



Nomenclature

AEG	acyl,alk(en)ylglycerol	Core lipid
CDP	cytidine diphosphate	
DAG	diacylglycerol	Core lipid
DEG	dietherglycerol	Core lipid
DGDG	diglycosyldiacylglycerol	Head group
DGTS	diacylglycerylhydroxymethyltrimethyl-(N,N,N)-homoserine	Head group
DMPE	phosphatidyl-(N,N)-dimethylethanolamine	Head group
DPG (cardiolipin)	diphosphatidylglycerol	Head group
MGDG	monoglycosyldiacylglycerol	Head group
MMPE	phosphatidyl-(N)-methylethanolamine	Head group
OL	ornithine lipid	Head group
PA	phosphatidic acid	Head group
PC	phosphatidylcholine	Head group
PE	phosphatidylethanolamine	Head group
PG	phosphatidylglycerol	Head group
PI	phosphatidylinositol	Head group
PL	phospholipid	
PS	phosphatidylserine	Head group
Sph	sphingolipid	Core lipid

ABSTRACT

Membrane lipids and their related acyl and/or alkyl moieties are important biomarkers for investigating the microbial community in environmental samples. Intact polar lipids, as major membrane components of microbes, allow to better understand soil living microbial composition by assessing their entire membrane lipid structures. Here we report on the profiling of more than 300 membrane-derived intact polar lipids in grassland soils differing in plant community and soil properties. In order to determine the origin of the soil lipid profiles we compared them to the intact polar lipids of plant roots, fungi, amoebae and Collembola. In addition, we investigated the impact of plant and soil related drivers on the composition of the soil lipid profiles. The soil lipid profiles were substantially more diverse and evenly distributed than the profiles of the soil-borne organisms studied. Moreover, the profiling analysis showed a substantial amount of odd-chain acyl species and low levels of unsaturation in intact polar lipids of soils. This suggests that the intact polar lipids in soil are primarily derived from bacteria, rather than plants, fungi or soil animals. The lipid composition was significantly affected by soil properties and plant diversity, emphasizing that the soil lipid profiles respond to abiotic and biotic environmental drivers. The plant diversity effect on the soil lipid profiles was mainly driven by changes in the N-methylation pathway of phosphatidylcholine in soil bacteria. This suggests that soil lipid profiles not only provide new insight into composition of microbial communities, but also can be used for assessing bacterial functioning in soils.

1. Introduction

Soil microbial communities play an important role in biogeochemical cycles such as decomposition of organic matter, the regulation of soil fertility and soil carbon dynamics (Rillig and Mummey, 2006; Falkowski et al., 2008; Berg, 2009; Fierer et al., 2011; Schmidt et al., 2011). Therefore, the analysis of soil microbial community structure has become a subject of growing interest in recent years. As essential components of all living cells, membrane lipids and their associated fatty acids or fatty alcohols have diverse structures

and can be used as biomarkers to provide information on the composition of soil microbial community.

Over the last decades, fatty acids derived from phospholipids (PLFA) were increasingly used as important markers to provide insights into the composition of microbial communities in soils (Frostegård et al., 1993; Zelles, 1997), since phospholipids are essential structural components of all microbial cellular membranes. PLFA analysis has many advantages. PLFA can be measured by GC/MS which offers a rapid and cost-effective way to assess shifts in community composition, microbial biomass and activity (Frostegård et al., 2011), and to elucidate microbial strategies related to environmental stress (Zelles, 1999). On the other hand, there also are limitations to the PLFA method. First, the use of PLFA to indicate a particular taxonomic group is not always possible, because many PLFAs are not group or species specific (Frostegård et al., 2011). Second, although phospholipids are the primary component of total lipids in microbes, other phosphorus-free lipids such as glycolipids and aminolipids also contribute to the microbial membrane structures (Sohlenkamp and Geiger, 2016) and thus can constitute a significant fraction of the fatty acids extracted as PLFA. Those lipids may significantly bias the information assessed by PLFA analysis since they have distinct metabolic pathways and origins. Moreover, the PLFA approach selects for lipids produced by Eukarya and Bacteria since Archaea do not produce fatty acids and even among the Bacteria, production of ether-bound lipids appears to be widespread among uncultivated soil and sediment bacteria (e.g., Weijers et al., 2006; Liu et al., 2010; Evans et al., 2017). By using HPLC/MS protocols, scientists can analyze intact polar membrane lipids (Rütters et al., 2002; Sturt et al., 2004). Intact polar membrane lipids offer a clear advantage to PLFAs since they are the major component of membrane structures of all living cells and can be taxonomically more specific than their nonpolar fatty acids (Sturt et al., 2004). This resides in their molecular structure (as the fatty acids or fatty alcohols are linked to the head groups) which offers higher source specificity and low molecular stability. Intact polar lipids are directly degraded after cell death, contrary to the fatty acids derived from PLFAs which can be preserved for several years (Schmidt et al., 2011). However, it is more difficult to define

the structures of fatty acids in intact polar lipids than to define them in direct analysis of fatty acids.

Intact polar lipids typically consist of two fatty acids/alcohols linked by a glycerol moiety to a variable head group (Fig. 1, Sohlenkamp and Geiger, 2016). The shape of intact polar lipids depends on both its head group type and chain length, and on the degree of unsaturation of the two acyl/ether chains (Cronan, 2003). Based on different head groups, intact polar lipids can be separated into two groups. One group consists of phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyl-(N)-methylethanolamine (MMPE), phosphatidyl-(N,N)-dimethylethanolamine (DMPE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylserine (PS), and others. The other includes phosphorus-free lipids comprising diacylglyceryl-(N,N,N)-trimethylhomoserine (DGTS), glycolipids (GL), sphingolipids (Sph), hopanoids (HOP), and others.

In plants, intact polar lipids play essential roles in photosynthesis, signal transfer, metabolism, plant growth, and response to external stresses (Welti et al., 2002; Welti and Wang, 2004). Intact polar lipids from microbes are in general similar to those of plants, but their composition, chain length and unsaturation of fatty acids/alcohols differ from that in plants. Based on head groups, microbial intact polar lipids can reflect microbial function, community composition, metabolic pathways, and environmental conditions (Benning et al., 1995; Russell et al., 1995; Zink et al., 2003; Schubotz et al., 2009; Rossel et al., 2011). Besides the classification of different head groups of membrane lipids, the sum of two fatty acid/alcohol chains of individual species (here we called molecular species, in the form of total acyl carbon:total double bonds) could be used to determine their origins like PLFAs. Many soil microbes restructure their membrane lipid composition in response to stress (López-Lara et al., 2005). For instance, bacterial phospholipids PC and PE can be replaced by phosphorus-free membrane lipids DGTS, GL and OL in response to phosphorus limitation (Geiger et al., 2010a; Geiger et al., 2010b; Geske et al., 2013). Microbes can respond to higher temperature by increasing their fatty acid chain length or decreasing the degree of unsaturation in their membrane

lipids (Zhang and Rock, 2008). Specific membrane lipid like PC have been found to be very important for direct plant-microbe interactions (López-Lara et al., 2003). Therefore, the information of intact polar lipid structures may provide a more detailed and holistic view of the microbial community structures in soils.

Until now, most studies of microbial intact polar lipids were carried out on pure cultures (Sohlenkamp and Geiger, 2016). However, the composition of bacteria in cultures may greatly differ from that in soils, due to the differences in nutrient availability and in bacterial community composition. In addition, the culturable bacteria represent only a small fraction of the total bacterial communities in nature (Stewart, 2012). Studies characterizing intact polar membrane lipids in soils and determining their sources are lacking; therefore, the comprehensive use of those markers to study microbial community structures and their functions in soils is restricted to a few studies (Schubotz et al., 2009; Borin et al., 2010).

To fill this gap, we studied full intact polar lipid profiles of soils of different grassland communities and compared them to lipid profiles of different soil-borne organisms or compartments (amoebae, fungi, Collembola and plant roots). Our study was conducted in a semi-natural grassland experiment with plant communities differing mainly in diversity (Roscher et al., 2004; Weisser et al., 2017). Specifically, we evaluated i) the differences between the composition of soil intact polar lipids and soil-borne organisms and ii) effects of environmental parameters (i.e., plant diversity and soil properties) on soil intact polar lipid composition.

2. Materials and Methods

2.1. Study site and sampling

Soil and root samples were taken at the Jena Experiment, a large grassland biodiversity experiment located in the Saale valley near Jena (east Thuringia, Germany, 50°55′N, 11°35′E, 130 m above sea level). In 2002, the experiment was established with a total number of 81 grassland plots of 20 × 20 m (Roscher et al., 2004). The soil type

is Eutric Fluvisol and the soil texture changes from sandy loam to silty clay with increasing distance to the Saale river (FAO-Unesco, 1997; Fischer et al., 2014). Along with the soil texture, other soil properties, such as pH (7.1–8.4) and the soil contents of organic carbon (5–33 g C kg^{-1}) and total nitrogen (1.0–2.7 g N kg^{-1}) vary. The variations in the soil properties are considered in the design of the experiment (Roscher et al., 2004). To account for the spatial variation of soil properties, the plots were arranged in four blocks. The established plant diversity gradient ranged from monocultures to communities with 60 species (1, 2, 4, 8 ,16 and 60). All 60 species in the plots are native in the Central European mesophilic grasslands. Further details about the classification of plant functional groups can be found in Roscher et al. (2004) and Weisser et al. (2017).

In June 2016, three surface soil samples (0−10 cm) from each plot were collected, combined to reduce the spatial heterogeneity, and homogenized. The soil samples were sieved (2 mm mesh size). Fine roots (if present) were picked using steel tweezers and stored at −20 ℃. The root samples were taken separately from six plots with different combinations of four functional groups (grass, legume, tall herb, small herb; Table S1). The roots were washed, freeze-dried and frozen.

2.2. Soil-borne reference organisms and their cultivation

All fungal strains used originate from Jena Microbial Resource Collection (JRMC), University of Jena and HKI, Germany. The saprotrophic fungi *Schizophyllum commune* FSU:3214xFSU:2896 and *Mucor plumbeus* JMRC:SF:013709 were cultivated in Petri dishes on solid complex yeast medium (CYM; Schwalb and Miles, 1967) and the mycorrhizal fungi *Tricholoma vaccinum* JMRC:FSU:4731 and *Pisolithus tinctorius* FSU:10019 on modified Melin Norkrans b (MMNb) medium (Kottke et al., 1987) at room temperature for 2 and 5 days for the fast growing *M. plumbeus* and *S. commune* and 2 and 3 weeks for the slow growing *P. tinctorius* and *T. vaccinum* (Table S1).

The Collembola species *Heteromurus nitidus* (Templeton, 1835) and *Folsomia candida* Willem, 1902 were taken from laboratory cultures fed with baker's yeast (*Saccharomyces cerevisiae*; Table S1). Laboratory cultures were maintained in glass jars filled with moist

potting soil at 15 °C in darkness and kept moist with distilled water. Before analysis, Collembola were starved for three days to empty their guts; subsequently they were frozen and stored in methanol.

Polysphondylium pallidum strain was from the Stallforth Lab at Leibniz Institute for Natural Product Research and Infection Biology in Jena (Germany). Amoebae were cultured (xenically) in the presence of the bacterium *Klebsiella aerogenes* as food. Briefly, amoebal spore suspension (from previously collected sori) was added to the surface of SM/5 agar plate seeded with 1 X 10^8 CFU/ml food bacterium *K. aerogenes*. Plates were incubated at 22°C for 7 to 10 days for mature fruiting bodies to appear. The entire cell mass of amoebal fruiting bodies was carefully collected using a sterile inoculation loop and suspended in KK2 buffer. This cell mass was washed clean of any attached bacteria using the same buffer. Resulting amoebal cells were then subjected to further analysis.

Before analyses, roots, fungi, Collembola and amoebae were frozen in liquid nitrogen. They were ground into fine powder and extracted using the same protocol as for the soil samples.

2.3. Lipid analysis

The detailed procedure for lipid extraction and analysis is described by Wörmer et al. (2015). In short, soil surface samples (~6 g) from the plots of the Jena Experiment were sequentially ultrasonically extracted with three different solvent mixtures; dichloromethane:methanol:phosphate buffer (1:2:0.8, v:v:v, 8.7 g L^{-1} KH_2PO_4, pH 7.4), dichloromethane:methanol:trichloroacetic acid buffer (1:2:0.8, v:v:v, 50 g L^{-1} CCl_3COOH, pH 2) and methanol:dichloromethane (5:1, v:v). Each solvent mixture was used twice, and the supernatants were combined in a separatory funnel. An equal amount of dichloromethane and water was added to the pooled extracts. After separation of the two layers, the organic (lower) phase was collected. The organic fractions were concentrated to near dryness on a rotary evaporator followed by a gentle stream of nitrogen, then transferred to small vials and stored at −20 ℃.

The analysis of intact polar lipids was carried out on a Dionex Ultimate 3000RS ultra high-performance liquid chromatography (UHPLC) instrument connected to a Bruker maXis Ultra-High Resolution quadrupole time-of-flight tandem mass spectrometer (Q-TOF) via an electrospray ion source (ESI). The total lipid extracts were dissolved in dichloromethane:methanol (9:1, v:v) and spiked with an internal standard (phosphatidylcholine, di21:0-PC). Intact polar lipid separation was achieved with an Acquity BEH Amide column (1.7 µm, 2.1 × 150 mm; Waters Corporation, Eschborn, Germany) at a constant flow rate of 0.4 mL min $^{-1}$. The solvent gradient was 99% A (acetonitrile:dichloromethane, 75:25, with 0.01% formic acid and NH_3, v:v:v:v) and 1% B (methanol:water, 50:50, with 0.4% formic acid and NH_3) for 2.5 min, then the amount of B increased from 5% at 5 min to 25% at 22.5 min, and then to 40% B in 4 min. The column was flushed with 40% B for 1 min. Parameters for the ion source and MS were those described by Wörmer et al. (2013). Identification of intact polar lipids was performed via the molecular ions, fragment ions resulting from MS^2 experiments in combination with general fragmentation rules for mass spectrometric analysis of intact polar lipids in positive ionization mode (Sturt et al., 2004), and relative retention times. Lipids were analyzed in the form of $[M+H]^+$ and $[M+NH_4]^+$ ions in the positive mode, while negative mode was also used to interpret the fatty acid side chains. Concentrations of intact polar lipids in each class were calculated by comparison with the internal standard (Wörmer et al., 2015). Response factors for each class of lipids were approximated by external calibration with standards: PC (di16:0), PE (di16:0), MMPE (di16:0), DMPE (di16:0), PG (di16:0), CL (tetra16:0), MGDG (di16:0), DGDG (16:0/18:0), DGTS (34:0) and glucosyl(ß)ceramide (18:0/d18:1). For the class of lipids with no standards available (e.g., ornithine lipids), a response factor from another class of amino lipid (DGTS) was used for calculation. Finally, concentrations of all the polar lipids are reported in ng g^{-1} dry weight soil after removing the soil moisture.

2.4. Statistical analyses

For a simple description of the lipid profiles we compared the lipid richness per sample and the dominance structure [as reciprocal Simpson's index (1/D) in the lipid

profile among groups (soil, roots, Collembola, fungi, amoebae)]. To test whether average values of richness and dominance structure among different groups were significantly different, we applied Kruskal-Wallis test followed by Mann-Whitney U tests to account for the unevenly distributed sample size among groups. For determining how the lipid composition varies among sample groups, we applied a principal component analysis (PCA). Furthermore, we evaluated the impact of environmental parameters and block effect on the intact polar lipid classes of the soil samples by applying redundancy analysis (RDA). Both PCA and RDA were performed with CANOCO 5.

3. Results

3.1. Distribution of intact polar lipid classes in soils

In soils, over 300 membrane intact polar lipids of 16 major classes (based on head groups) were identified (Table 1, Figs. 2 and S1). These classes included phospholipids (PC, PE, MMPE, DMPE, PG, PI, CL and related single-chained classes lysoPC, lysoPE, lysoMMPE, lysoDMPE and lysoPG), aminolipids (DGTS and OL), and glycolipids (MGDG and DGDG). All phospholipid and glycolipid classes were found to contain core lipid diacylglycerols (DAG), while dietherglycerol (DEG) and sphingosine (Sph) were only found with the polar head groups PC (PC-DEG and PC-Sph, Fig. 2 and S1). Total intact polar lipid concentration in all soil samples varied between 5 and 187 $\mu g\, g^{-1}$ dry weight soil (dws). The most abundant intact polar lipid classes in all soil samples were PC with 28.3%, MGDG with 16.5% and PE with 14.0% of the total polar lipids (Table 1). Besides, OL, DGTS and PG were also abundant, accounting for 9.2%, 6.8% and 6.2% of the total polar lipids, respectively. Minor intact polar lipid classes (< 4%) included lysophospholipids (lysoPL), MMPE, DMPE, DGDG, DPG, PC-Sph and PC-DEG. The membrane lipid classes, DMPE, lysoDMPE, DPG, PC-Sph, PC-DEG and OL, were only found in soils and not in plant roots, fungi and Collembola. The relative distribution of most intact polar lipids in soils changed little between plots and blocks in the Jena Experiment (Table 1, Fig. 3). PG varied substantially between blocks (SD = 3.8%), with a contribution of 10% in block 4 but only 3% in block 1.

Molecular species (defined in the introduction; Fig. 1) in each lipid class of the surface soils and other organisms are shown in Fig. 4 and S2-S3. In soils, among all phospholipid classes, PE comprised the smallest range of acyl species lengths with total numbers of carbon atoms varying between 30 to 34, while the other phospholipids PC, MMPE, DMPE and PG contained molecular species ranging from 31 to 38 (Fig. 4).

3.2. Differences of lipid profiles between soil and soil-borne organisms

Total intact polar lipid concentrations in all the soil-borne organisms (plant roots, fungi, Collembola and ameobae) were two to four magnitudes higher than those in soils (Table 1). However, molecular species richness and abundance distribution was considerably higher in soils than in other organisms. Notably, lipid molecular species with odd number carbon chains were found only in soils (Fig. 2), accounting for 31% of total soil lipids. PC (35:1), PC (37:2) and PE (33:1) were the most abundant odd number carbon molecular species in soils, comprising 1.3%, 3.0% and 2.1% of the total lipid pool (Fig. 4). In this work, the carbon numbers refer to total acyl carbons and the "double bond number" refers to double bond equivalents, which could be double bonds or ring, such as cyclopropane rings.

In plant roots, around 140 types of membrane lipids were found and quantified. Different from lipids in soils, MGDG was the most dominant lipid class in root samples, accounting for 44.3% of the total polar lipids (Table 1, Fig. 2). Another type of glycolipid, DGDG comprised 5.9% of total polar lipids. Phospholipids PC, PE, PG, lysoPC and lysoPE accounted for a significant amount of the total membrane lipids in roots (> 5%, respectively). Fungi and Collembola in our study contained less than 100 types of membrane lipids. Fungi consisted of large amounts of phospholipids PC, PE, lysoPC and lysoPE (in total >80%), while Collembola and amoebae had only three different types of membrane lipids that were PC, PE and lysoPC.

In the fraction of phospholipid classes (Fig. 4), the major molecular species of PC of soils was 36:2 (18:1/18:1, Table 2), representing 17% of the total PC. The molecular species of all other phospholipids were randomly distributed in soils. Plant roots, fungi and

Collembola generally comprised phospholipids with 34 and 36 carbon atoms and 2 to 6 double bonds, whereas phospholipids of soils contained 0 to 2 double bonds and molecular species with one or two double bonds were most abundant. In the fraction of lysophospholipid classes (Fig. S2), lysophospholipids of soils possessed mostly 15 to 19 carbon acyl species without double bonds. The molecular species patterns of lysoPC and lysoPE were similar, while lysoMMPE only contained long chain fatty acids (number of carbons 18 to 21). Plant roots and fungi contained considerable amounts of 18:1 and 18:2 polar lipids. In addition, 20:1 to 20:3 were exclusively present in plants. In the fraction of non-phosphorus lipid classes (Fig. S3), double bonds of molecular species in soils also varied between 0 to 2 except for DGDG. DGDG of soils mainly consisted of 32, 34 and 36 carbon species with unsaturation from 0 up to 6. Plant roots contained large amounts of MGDG (34:2) and MGDG (36:2 to 36:6). In soils, the dominant species of aminolipids DGTS and OL were 34:1 and 39:1, respectively. Fungi comprised substantial amounts of DGTS (34:2), DGTS (36:2) and DGTS (38:2).

Principal component analysis (PCA) indicated significant differences in the relative abundances of all the membrane intact polar lipid classes of soils and other soil-borne organisms. The first principal component (PC1) explained 60.0% of the variance of all the lipid classes and the second principal component (PC2) explained 18.5% (Fig. 3). Phospholipids PC, PE, lysoPC and lysoPE were related to each other along the positive direction of the first principle component, whereas all the other lipids were shown in the negative direction. Glycolipids MGDG, DGDG and phospholipids PE, PG, lysoPC and lysoPE correlated positively with the second principal component. When considering sample distribution, the soil group differed clearly from all the soil-borne organisms.

3.3. Effects of environmental parameters on soil lipid profiles

Redundancy Analysis (RDA) revealed significant effects of abiotic and biotic parameters on the composition of soil intact polar lipids. The abiotic soil parameters (tested as block, see Materials and Methods) explained 17.5% ($P = 0.002$), while plant diversity (tested as plant species richness) explained 5.0% ($P = 0.006$) of the variation in the composition of intact polar lipids (Fig. 5). Block correlated positively with PG

groups (PG, lysoPG and CL) and glycolipid MGDG. Plant diversity had a positive effect on MMPE, DMPE and MGDG, but a negative effect on the composition of PC and PE.

4. Discussion

4.1. Bacteria as major sources of intact polar lipids in soils

The distribution of lipid classes in soils differed clearly from all the soil-borne organisms (plant roots, fungi, Collembola and amoebae) tested in this study, suggesting intact polar lipids in soils originate from different sources. Intact polar lipids were much more diverse in soils than in plant roots, fungi, Collembola and amoebae. Furthermore, the concentrations of lipid molecular species in soils were more evenly distributed than those in the studied organisms. The dominant lipid classes found in soils were PC, PE, PG, DGTS and MGDG. PE, PG and cardiolipin are the major structural constituents of most bacterial membranes (Gill, 1975; Raetz and Dowhan, 1990). In addition, there is a large diversity of membrane lipids in bacteria (López-Lara et al., 2003; Sohlenkamp et al., 2003). DGTS occurs in lower plants, algae, bryophytes, fungi as well as in photosynthetic bacteria (Eichenberger, 1993; Benning et al., 1995; Cañavate et al., 2016). Phosphatidylcholine (PC) is one of the key components of eukaryotic membranes but may also occur in ca. 15% in all bacterial species (Sohlenkamp et al., 2003). However, the high amount of PC (28.3%) and the small amount of PG (6.2%) in our soil samples likely reflect eukaryotic rather than bacterial origin. However, to our knowledge, most studies of bacterial membrane polar lipids are carried out in cultured conditions but not in natural environments which may lead to a bias view on lipid distribution in microorganisms. Higher abundance of PC than PG produced by bacteria was also reported from permafrost soils in Svalbard (Rethemeyer et al., 2010), suggesting that bacteria adjust the membrane lipid composition of their community in different environments (Zhang and Rock, 2008). Therefore, the majority of lipids found in the studied soils presumably originated from soil microbes.

Plants commonly synthesize intact polar lipids containing molecular species with even carbon numbers, particularly 16 and 18 (Devaiah et al., 2006). These molecular species of lipids are non-randomly distributed and contain high degrees of unsaturation,

typically up to three double bonds per chain, with 2-3 the most common in 18C plant lipids (Welti et al., 2002; Welti and Wang, 2004). This trend was also seen in our plant root samples. In contrast, bacteria synthesize high quantities of intact polar lipid acyl chains with both even and odd carbon numbers, from 14 to 20, typically with zero to one double bond (Shaw, 1974; Řezanka and Sigler, 2009). In our soil samples, lipid species with odd carbon numbers accounted for 31% of total, similar to the amount of odd carbon chain PLFA measured in cultured bacteria (Zelles, 1997). From the well-studied membrane lipid group of PLFAs it is known that odd carbon chain were primarily found in bacteria rather than in fungi and plants (Zelles, 1997). Here the large proportion of phospholipid odd number molecular species with low unsaturation (0 to 2 per phospholipid, or 0 to 1 per fatty acid) in soils indicates bacterial rather than plant and fungal origin. The PLFA linoleic acid (18:2$w6$) has been considered as reliable indicator for fungal biomass (Frostegård et al., 2011). In agreement with a major bacterial origin of the intact lipids in soils, both PC (36:4) and PE (36:4) were the most abundant molecular species in our fungi samples, although we could not identify the position of double bonds in acyl species, but they were not detected in soils. In addition, the degree of structural variability of lysophospholipids in our soil samples surpassed that in plant roots, fungi and Collembola. In the soil samples acyl carbon number ranged from 13 to 20 and had less than two double bonds per acyl chain, further confirming bacteria as major sources of intact polar lipids in soils.

Commonly, higher plants are characterized by a high proportion of glycolipids, MGDG (monogalactosyldiacylglycerol) and DGDG (digalactosyldiacylglycerol), with 34 to 36 acyl chains and 6 double bonds (Wang et al., 2006). A similar pattern was found in our plant root samples. Soils contained trace amounts of highly unsaturated DGDG molecular species, suggesting small contributions of plant-derived lipids to soils, maybe due to root residues. However, MGDG in soils contained no more than one double bond, suggesting they consist a headgroup of glucose instead of galactose and they are probably coming from bacteria (Hölzl and Dörmann, 2007). In fact, the photosynthetic bacteria *Rhodobacterales*, *Rhodospirillales*, *Rhizobiales* and non-photosynthetic

bacteria *Firmicutes* can synthesize such types of MGDG (Hölzl and Dörmann, 2007), suggesting they form the source of these glycolipids. Supporting bacterial origin of those lipids, a substantial abundance of OL, which has been linked with aerobic phototrophic bacteria, e.g., *Rhodobacterales*, was also found in soils (Aygun-Sunar et al., 2006; Zhang et al., 2009).

4.2. Impacts of environmental parameters on the composition of intact polar lipids in soils

Significant differences of intact polar lipid composition were found between blocks, representing different soil properties, suggesting that soil texture is a major driver regulating bacterial lipid distributions in soil. Microbial community composition has been shown to be influenced by soil properties in several studies (Sessitsch et al., 2001; de Vries et al., 2012; Dassen et al., 2017). In addition, high diversity of plant communities increased the production of the intermediates (MMPE and DMPE) in the N-methylation pathway but decreased the amount of two end products, PC and PE. This suggests that plant diversity affects the N-methylation pathway of phosphatidylcholine in soil bacteria. There are two metabolic pathways to biosynthesize PC, the CDP-choline pathway and the phospholipid N-methylation pathway (Kent, 1995; Sohlenkamp and Geiger, 2016). The N-methylation pathway is the primary route for PC formation in bacteria, yeast and other fungi (Kodaki and Yamashita, 1987; López-Lara and Geiger, 2001). Some specific bacteria could produce PC using the choline obtained from their association with eukaryotes/plants using the CDP-choline pathway, e.g., *Sinorhizobium meliloti* and *Agrobacterium tumefaciens* (Sohlenkamp et al., 2003). In the N-methylation pathway, PE is methylated three consecutive times via intermediate products as MMPE and DMPE to PC by the N-methyltransferase enzyme. This N-methylation pathway for PC synthesis, as indicated by levels of MMPE and DMPE, was found to be mediated by plant diversity in our experiment. The N-methylation pathway is found in pathogens, e.g., *Agrobacterium tumefaciens* and *Legionella pneumophila* (Conover et al., 2008; Moser et al., 2014) as well as in some nitrogen-fixing bacteria, e.g., *Sinorhizobium melilotii*, *Bradyrhizobium japonicum* and *Rhodobacter sphaeroides* (Arondel et al.,

1993; de Rudder et al., 1997; De Rudder et al., 2000). Eisenhauer et al. (2012) found species-rich plant communities to have higher levels of nitrogen availability and less pathogen pressure than species-poor ones, because there were more heterogeneous and diverse soil microbial communities. The negative correlation between PC and plant diversity may indicate less pathogen pressure and higher facilitative effects (e.g., nitrogen fixation) of soil microbes in response to the higher plant diversity. As MGDG was found to positively correlate with plant species richness, plant diversity may also enhance the community of photosynthetic bacteria.

5. Conclusion

We provide a comprehensive intact polar lipid profiling with over 300 glycerolipid species in grassland soils. The comparison of these soil lipid profiles with various soil-borne organisms strongly suggests that these lipids mainly originate from bacteria. Many soil bacteria are difficult to culture and additionally bacteria adjust their membrane lipid compositions as a function of the environment. Therefore, their communities and compositions are more complex in natural environments than in cultures. Our data emphasize the importance of environmental parameters on the composition of soil microbial membrane lipids, potentially affecting microbial metabolic pathways and functions. Compared to PLFA analysis, profiling of polar lipids provides more information on the function of different lipid classes with head groups. The determination of intact polar lipids from soils thus offers the possibility to better understand the structure and functioning of soil microbial communities by studying their intact membrane lipid structures. As the function of these lipids still is little understood, more data are needed on the intact polar lipid composition of microorganisms, in particular bacteria, in soils.

Acknowledgements

We thank Steffen Ruehlow, Maria Foerster, Stefanie Albrecht, Odette Gonzalez for their help during soil sampling and lipid extraction. Prof. Georg Pohnert, Prof. Ernst Heinz and Prof. Christian Sohlenkamp gave valuable comments on early lipid data analysis.

We thank all the people that were involved in planning, set up and maintaining of the experiment and the German Research Foundation (DFG) for financial support of FOR 456, FOR 1451 and Gl262-14. The data on the individual intact polar lipids has been deposited in the Pangaea database (https://pangaea.de/) under doi: https://issues.pangaea.de/browse/PDI-19785.

References

Arondel, V., Benning, C., Somerville, C.R., 1993. Isolation and functional expression in Escherichia coli of a gene encoding phosphatidylethanolamine methyltransferase (EC 2.1.1.17) from Rhodobacter sphaeroides. Journal of Biological Chemistry 268, 16002-16008.

Aygun-Sunar, S., Mandaci, S., Koch, H.G., Murray Ian, V.J., Goldfine, H., Daldal, F., 2006. Ornithine lipid is required for optimal steady-state amounts of c-type cytochromes in Rhodobacter capsulatus. Molecular Microbiology 61, 418-435.

Benning, C., Huang, Z.H., Gage, D.A., 1995. Accumulation of a novel glycolipid and a betaine lipid in cells of Rhodobacter sphaeroides grown under phosphate limitation. Archives of Biochemistry and Biophysics 317, 103-111.

Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology 84, 11-18.

Borin, S., Ventura, S., Tambone, F., Mapelli, F., Schubotz, F., Brusetti, L., Scaglia, B., D'Acqui, L.P., Solheim, B., Turicchia, S., Marasco, R., Hinrichs, K.-U., Baldi, F., Adani, F., Daffonchio, D., 2010. Rock weathering creates oases of life in a High Arctic desert. Environmental Microbiology 12, 293-303.

Cañavate, J.P., Armada, I., Ríos, J.L., Hachero-Cruzado, I., 2016. Exploring occurrence and molecular diversity of betaine lipids across taxonomy of marine microalgae. Phytochemistry 124, 68-78.

Conover, G.M., Martinez-Morales, F., Heidtman, M.I., Luo, Z.-Q., Tang, M., Chen, C., Geiger, O., Isberg, R.R., 2008. Phosphatidylcholine synthesis is required for

optimal function of Legionella pneumophila virulence determinants. Cellular Microbiology 10, 514-528.

Cronan, J.E., 2003. Bacterial membrane Lipids: where do we stand? Annual Review of Microbiology 57, 203-224.

Dassen, S., Cortois, R., Martens, H., de Hollander, M., Kowalchuk, G.A., van der Putten, W.H., De Deyn, G.B., 2017. Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. Molecular Ecology 26, 4085-4098.

de Rudder, K.E., Thomas-Oates, J.E., Geiger, O., 1997. Rhizobium meliloti mutants deficient in phospholipid N-methyltransferase still contain phosphatidylcholine. Journal of Bacteriology 179, 6921-6928.

De Rudder, K.E.E., López-Lara, I.M., Geiger, O., 2000. Inactivation of the gene for phospholipid N-methyltransferase in Sinorhizobium meliloti: phosphatidylcholine is required for normal growth. Molecular Microbiology 37, 763-772.

de Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H.C., Kattge, J., Bardgett, R.D., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters 15, 1230-1239.

Devaiah, S.P., Roth, M.R., Baughman, E., Li, M., Tamura, P., Jeannotte, R., Welti, R., Wang, X., 2006. Quantitative profiling of polar glycerolipid species from organs of wild-type Arabidopsis and a PHOSPHOLIPASE Dα1 knockout mutant. Phytochemistry 67, 1907-1924.

Eichenberger, W., 1993. Betaine lipids in lower plant. Distribution of DGTS, DGTA and phospholipids, and the intracellular localization and site of biosynthesis of DGTS. Plant Physiology and Biochemistry 31, 213-221.

Eisenhauer, N., Reich, P.B., Scheu, S., 2012. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. Basic and Applied Ecology 13, 571-578.

Evans, T.W., Wörmer, L., Lever, M.A., Lipp, J.S., Lagostina, L., Lin, Y.-S., Jørgensen, B.B., Hinrichs, K.-U., 2017. Size and composition of subseafloor microbial

community in the Benguela upwelling area examined from intact membrane lipid and DNA analysis. Organic Geochemistry 111, 86-100.

Fahy, E., Subramaniam, S., Murphy, R.C., Nishijima, M., Raetz, C.R.H., Shimizu, T., Spener, F., van Meer, G., Wakelam, M.J.O., Dennis, E.A., 2009. Update of the LIPID MAPS comprehensive classification system for lipids. Journal of Lipid Research 50, 9-14.

Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The microbial engines that drive earth's biogeochemical cycles. Science 320, 1034-1039.

FAO-Unesco, 1997. Soil map of the world : revised legend with corrections and updates. ISRIC, Wageningen.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2011. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME journal 6, 1007-1017.

Fischer, C., Roscher, C., Jensen, B., Eisenhauer, N., Baade, J., Attinger, S., Scheu, S., Weisser, W.W., Schumacher, J., Hildebrandt, A., 2014. How do earthworms, soil texture and plant composition affect infiltration along an experimental plant diversity gradient in grassland? PLOS ONE 9, e98987.

Frostegård, Å., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723-730.

Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43, 1621-1625.

Geiger, O., González-Silva, N., López-Lara, I.M., Sohlenkamp, C., 2010a. Amino acid-containing membrane lipids in bacteria. Progress in Lipid Research 49, 46-60.

Geiger, O., Sohlenkamp, C., López-Lara, I.M., 2010b. Formation of bacterial membrane lipids: pathways, enzymes, reactions, In: Timmis, K.N. (Ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 395-407.

Geske, T., vom Dorp, K., Dörmann, P., Hölzl, G., 2013. Accumulation of glycolipids and other non-phosphorous lipids in Agrobacterium tumefaciens grown under phosphate deprivation. Glycobiology 23, 69-80.

Gill, C., 1975. Effect of growth temperature on the lipids of Pseudomonas fluorescens. Microbiology 89, 293-298.

Hölzl, G., Dörmann, P., 2007. Structure and function of glycoglycerolipids in plants and bacteria. Progress in Lipid Research 46, 225-243.

Kent, C., 1995. Eukaryotic phospholipid biosynthesis. Annual Review of Biochemistry 64, 315-343.

Kodaki, T., Yamashita, S., 1987. Yeast phosphatidylethanolamine methylation pathway. Cloning and characterization of two distinct methyltransferase genes. Journal of Biological Chemistry 262, 15428-15435.

Kottke, I., Guttenberger, M., Hampp, R., Oberwinkler, F., 1987. An in vitro method for establishing mycorrhizae on coniferous tree seedlings. Trees 1, 191-194.

López-Lara, I.M., Gao, J.-L., Soto, M.J., Solares-Pérez, A., Weissenmayer, B., Sohlenkamp, C., Verroios, G.P., Thomas-Oates, J., Geiger, O., 2005. Phosphorus-free membrane lipids of Sinorhizobium meliloti are not required for the symbiosis with Alfalfa but contribute to increased cell yields under phosphorus-limiting conditions of growth. Molecular Plant-Microbe Interactions 18, 973-982.

López-Lara, I.M., Geiger, O., 2001. Novel pathway for phosphatidylcholine biosynthesis in bacteria associated with eukaryotes. Journal of biotechnology 91, 211-221.

López-Lara, I.M., Sohlenkamp, C., Geiger, O., 2003. Membrane lipids in plant-associated bacteria: their biosyntheses and possible functions. Molecular Plant-Microbe Interactions 16, 567-579.

Liu, X.-L., Leider, A., Gillespie, A., Gröger, J., Versteegh, G.J.M., Hinrichs, K.-U., 2010. Identification of polar lipid precursors of the ubiquitous branched GDGT orphan lipids in a peat bog in Northern Germany. Organic Geochemistry 41, 653-660.

Moser, R., Aktas, M., Narberhaus, F., 2014. Phosphatidylcholine biosynthesis in X anthomonas campestris via a yeast-like acylation pathway. Molecular Microbiology 91, 736-750.

Rütters, H., Sass, H., Cypionka, H., Rullkötter, J., 2002. Microbial communities in a Wadden Sea sediment core—clues from analyses of intact glyceride lipids, and released fatty acids. Organic Geochemistry 33, 803-816.

Raetz, C., Dowhan, W., 1990. Biosynthesis and function of phospholipids in Escherichia coli. Journal of Biological Chemistry 265, 1235-1238.

Rethemeyer, J., Schubotz, F., Talbot, H.M., Cooke, M.P., Hinrichs, K.-U., Mollenhauer, G., 2010. Distribution of polar membrane lipids in permafrost soils and sediments of a small high Arctic catchment. Organic Geochemistry 41, 1130-1145.

Řezanka, T., Sigler, K., 2009. Odd-numbered very-long-chain fatty acids from the microbial, animal and plant kingdoms. Progress in Lipid Research 48, 206-238.

Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytologist 171, 41-53.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W.W., Schmid, B., Schulze, E.-D., 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic and Applied Ecology 5, 107-121.

Rossel, P.E., Elvert, M., Ramette, A., Boetius, A., Hinrichs, K.-U., 2011. Factors controlling the distribution of anaerobic methanotrophic communities in marine environments: Evidence from intact polar membrane lipids. Geochimica et Cosmochimica Acta 75, 164-184.

Russell, N.J., Evans, R.I., ter Steeg, P.F., Hellemons, J., Verheul, A., Abee, T., 1995. Membranes as a target for stress adaptation. International Journal of Food Microbiology 28, 255-261.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Schubotz, F., Wakeham, S.G., Lipp, J.S., Fredricks, H.F., Hinrichs, K.-U., 2009. Detection of microbial biomass by intact polar membrane lipid analysis in the water column

and surface sediments of the Black Sea. Environmental Microbiology 11, 2720-2734.

Schwalb, M.N., Miles, P.G., 1967. Morphogenesis of Schizophyllum commune. II. Effect of microaerobic growth. Mycologia 59, 610-622.

Sessitsch, A., Weilharter, A., Gerzabek, M.H., Kirchmann, H., Kandeler, E., 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Applied and Environmental Microbiology 67, 4215-4224.

Shaw, N., 1974. Lipid composition as a guide to the classification of bacteria, In: Perlman, D. (Ed.), Advances in Applied Microbiology. Academic Press, pp. 63-108.

Sohlenkamp, C., Geiger, O., 2016. Bacterial membrane lipids: diversity in structures and pathways. FEMS Microbiology Reviews 40, 133-159.

Sohlenkamp, C., López-Lara, I.M., Geiger, O., 2003. Biosynthesis of phosphatidylcholine in bacteria. Progress in Lipid Research 42, 115-162.

Stewart, E.J., 2012. Growing unculturable bacteria. Journal of Bacteriology 194, 4151–4160.

Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology. Rapid Communications in Mass Spectrometry 18, 617-628.

Templeton, R., 1835. Thysanurae hibernicae. Trans. Ent. Soc. Lond 1, 89-98.

Wörmer, L., Lipp, J.S., Hinrichs, K.-U., 2015. Comprehensive Analysis of Microbial Lipids in Environmental Samples Through HPLC-MS Protocols, In: McGenity, T.J., Timmis, K.N., Nogales, B. (Eds.), Hydrocarbon and Lipid Microbiology Protocols: Petroleum, Hydrocarbon and Lipid Analysis. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 289-317.

Wörmer, L., Lipp, J.S., Schröder, J.M., Hinrichs, K.-U., 2013. Application of two new LC–ESI–MS methods for improved detection of intact polar lipids (IPLs) in environmental samples. Organic Geochemistry 59, 10-21.

Wang, X., Li, W., Li, M., Welti, R., 2006. Profiling lipid changes in plant response to low temperatures. Physiologia Plantarum 126, 90-96.

Weijers, J.W.H., Schouten, S., Hopmans, E.C., Geenevasen, J.A.J., David, O.R.P., Coleman, J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. Environmental Microbiology 8, 648-657.

Weisser, W.W., Roscher, C., Meyer, S.T., Ebeling, A., Luo, G., Allan, E., Beßler, H., Barnard, R.L., Buchmann, N., Buscot, F., Engels, C., Fischer, C., Fischer, M., Gessler, A., Gleixner, G., Halle, S., Hildebrandt, A., Hillebrand, H., de Kroon, H., Lange, M., Leimer, S., Le Roux, X., Milcu, A., Mommer, L., Niklaus, P.A., Oelmann, Y., Proulx, R., Roy, J., Scherber, C., Scherer-Lorenzen, M., Scheu, S., Tscharntke, T., Wachendorf, M., Wagg, C., Weigelt, A., Wilcke, W., Wirth, C., Schulze, E.-D., Schmid, B., Eisenhauer, N., 2017. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. Basic and Applied Ecology 23, 1-73.

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H.-E., Rajashekar, C.B., Williams, T.D., Wang, X., 2002. Profiling membrane lipids in plant stress responses: Role of phospholipase Dα in freezing-induced lipid changes in arabidopsis. Journal of Biological Chemistry 277, 31994-32002.

Welti, R., Wang, X., 2004. Lipid species profiling: a high-throughput approach to identify lipid compositional changes and determine the function of genes involved in lipid metabolism and signaling. Current Opinion in Plant Biology 7, 337-344.

Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35, 275-294.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111-129.

Zhang, X., Ferguson-Miller, S.M., Reid, G.E., 2009. Characterization of ornithine and glutamine lipids extracted from cell membranes of Rhodobacter sphaeroides. Journal of the American Society for Mass Spectrometry 20, 198-212.

Zhang, Y.-M., Rock, C.O., 2008. Membrane lipid homeostasis in bacteria. Nature Reviews Microbiology 6, 222-233.

Zink, K.-G., Wilkes, H., Disko, U., Elvert, M., Horsfield, B., 2003. Intact phospholipids—microbial "life markers" in marine deep subsurface sediments. Organic Geochemistry 34, 755-769.

Figure and Table captions

Fig. 1. Chemical structures of intact polar lipids modified from Wörmer et al. (2015), including head groups (left) and core lipids with glycerol backbone (right). Dashed lines mean position and number of carbons can change. Molecular species are the sum of two carbon chains. Lyso phospholipid only contain one carbon chain. Abbreviations: DAG, diacylglycerol; DEG, dietherglycerol; AEG, acyletherglycerol; DGTS, diacylglycerylhydroxymethyltrimethyl-(N,N,N)-homoserine; OL, ornithine lipid; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; MMPE, phosphatidyl-(N)-methylethanolamine; DMPE, phosphatidyl-(N,N)-dimethylethanolamine; Sph, sphingolipid; MGDG, monoglycosyldiacylglycerol; DGDG, diglycosyldiacylglycerol; OH, monohydroxy; Me, methylated.

Fig. 2. Richness of lipid compounds, lipid abundance distribution and carbon number of molecular species in soils (n = 76), plant roots (n = 6), fungi (n = 11), Collembola (n = 6) and amoebae (n = 1). The data averaged among different species/samples and the n represents species/samples used for the lipid measurement and data analysis.

Fig. 3. Unconstrained PCA based on the fractional abundances of intact polar lipid classes of the soils (n = 76), plant roots (n = 6), fungi (n = 11), Collembola (n = 6) and amoebae (n = 1).

Fig. 4. Molecular species of phopholipid classes (weight% of total intact polar lipids analyzed) in the soils (n = 76), plant roots (n = 6), fungi (n = 11) and Collembola (n = 6). The data averaged among different species/samples and the n represents species/samples used for the lipid measurement and data analysis. Values are means ± SD. Individual molecular species in each lipid class was shown in the type of total acyl carbons:total double bonds (Fahy et al., 2009). Only the most abundant molecular species are shown here.

Fig. 5. Redundancy analysis of plant species richness and block effect on the composition of intact polar lipids in soils. Abbreviation: PSR-plant species richness, Block-Block effect.

Fig. 1.

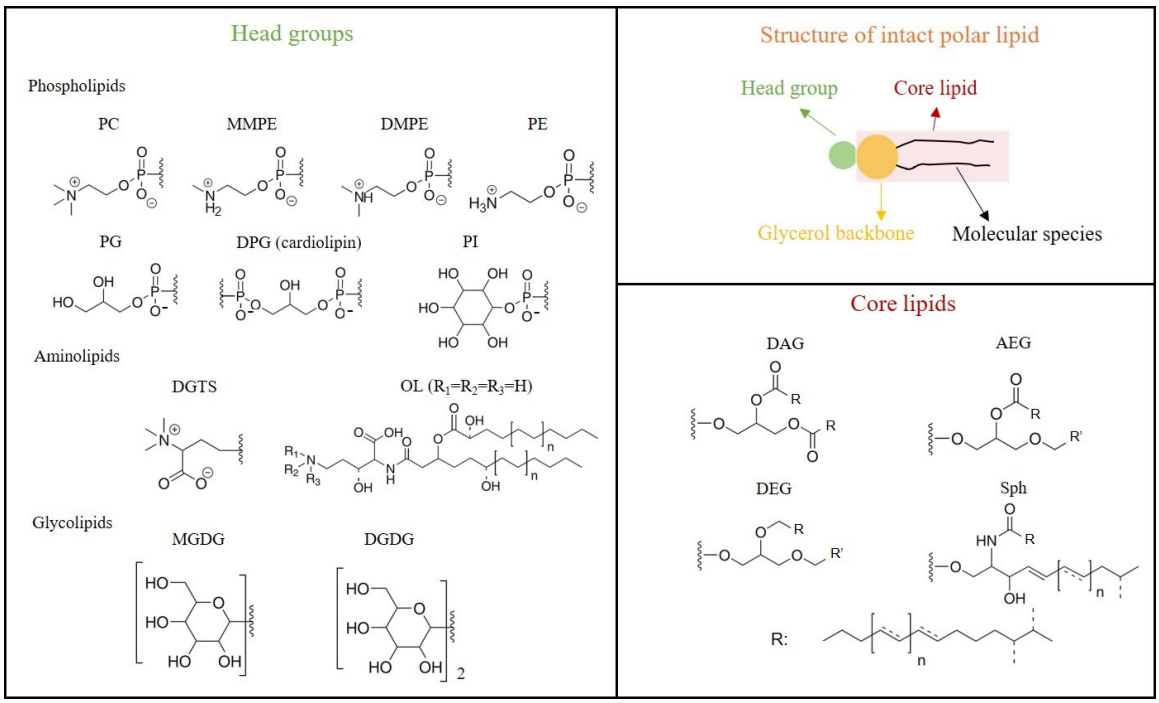

Head groups
Phospholipids
PC
MMPE
DMPE
PE
PG
DPG (cardiolipin)
PI
Aminolipids
DGTS
OL (R1=R2=R3=H)
Glycolipids
MGDG
DGDG
Structure of intact polar lipid
Head group
Core lipid
Glycerol backbone
Molecular species
Core lipids
DAG
AEG
DEG
Sph
R:

Fig. 2.

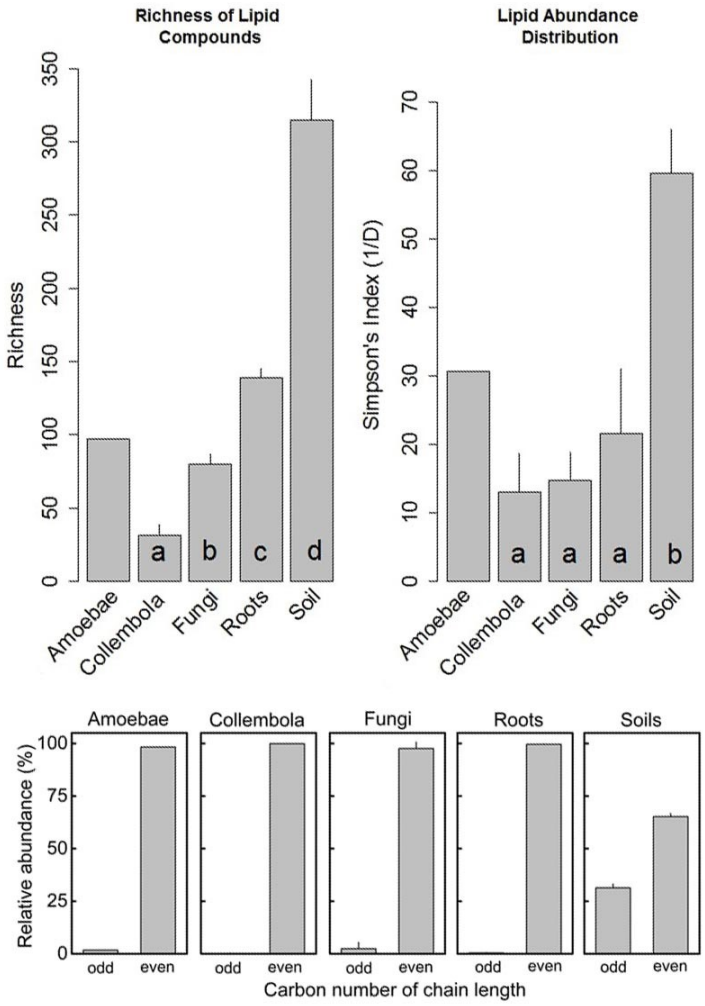

Richness of Lipid Compounds
Richness
0
50
100
150
200
250
300
350
a
b
c
d
Amoebae
Collembola
Fungi
Roots
Soil
Lipid Abundance Distribution
Simpson's Index (1/D)
0
10
20
30
40
50
60
70
a
a
a
b
Amoebae
Collembola
Fungi
Roots
Soil
Amoebae
Collembola
Fungi
Roots
Soils
Relative abundance (%)
0
25
50
75
100
odd
even
Carbon number of chain length

Fig. 3.

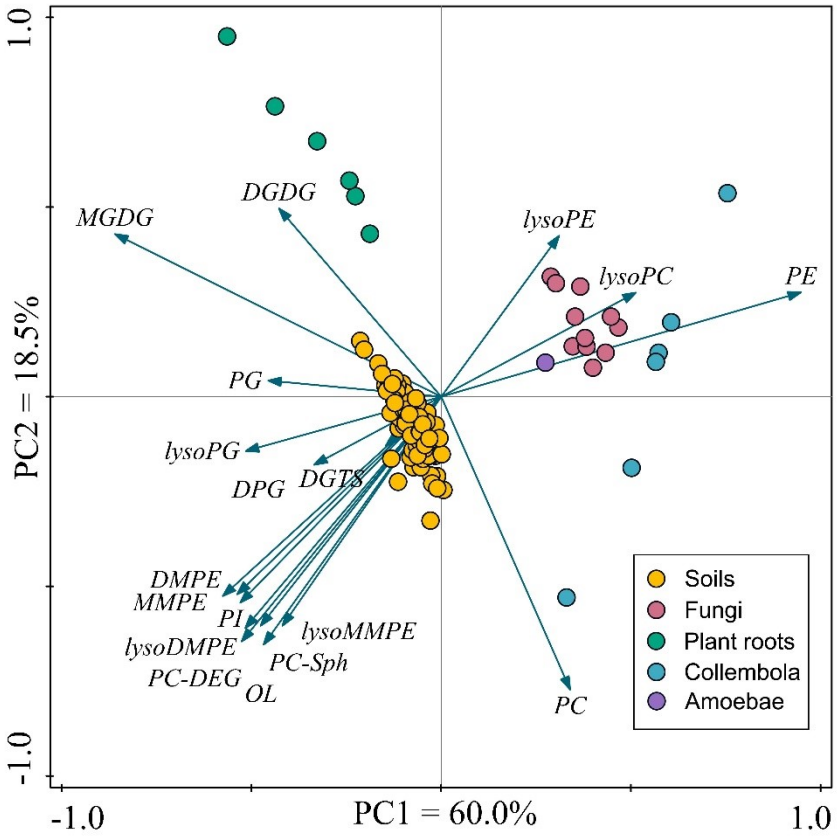

1.0
-1.0
PC2 = 18.5%
-1.0
PC1 = 60.0%
1.0
MGDG
DGDG
lysoPE
lysoPC
PE
PG
lysoPG
DPG
DGTS
DMPE
MMPE
PI
lysoDMPE
PC-DEG
OL
lysoMMPE
PC-Sph
PC
Soils
Fungi
Plant roots
Collembola
Amoebae

Fig. 4.

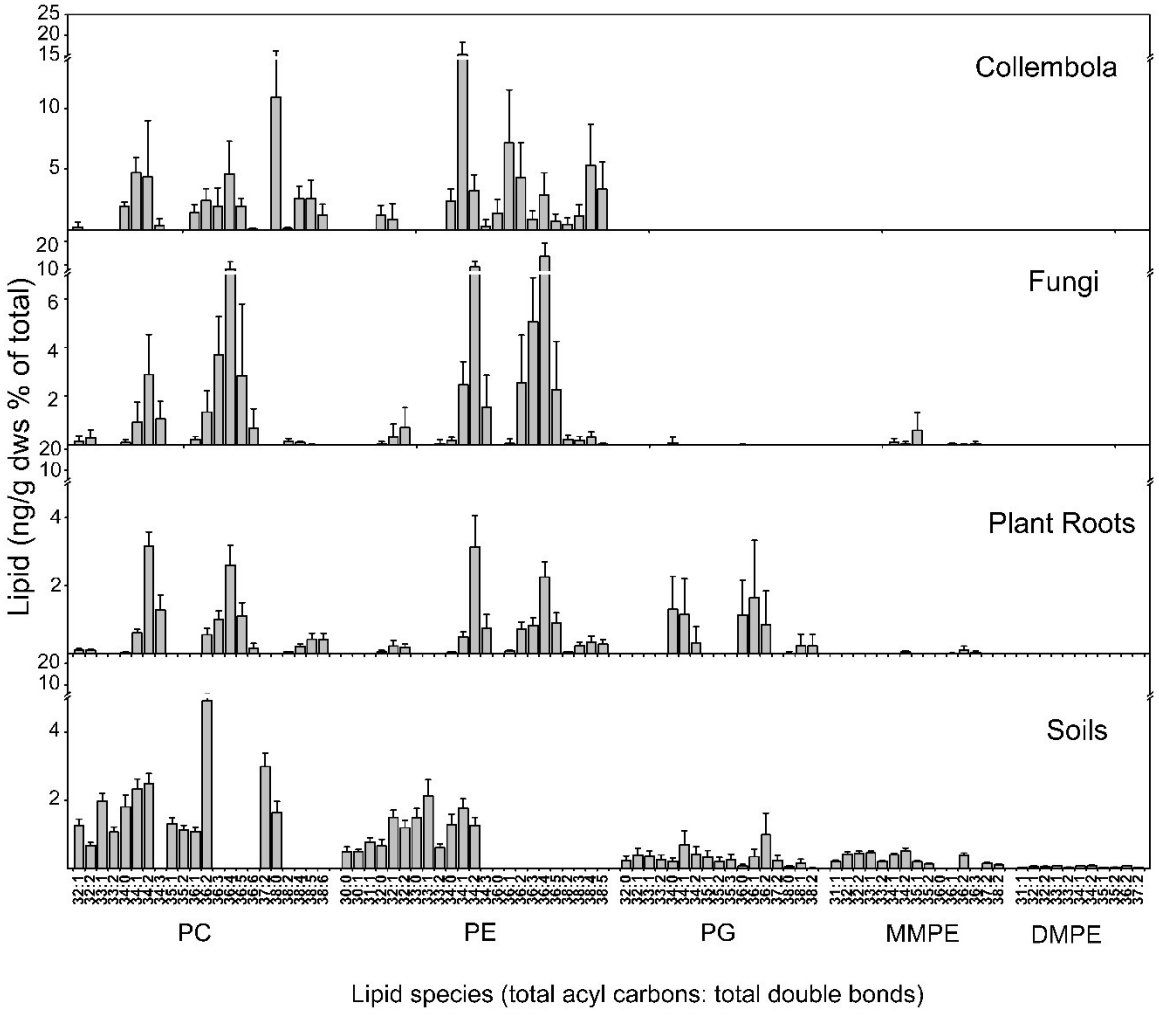

Collembola
Fungi
Plant Roots
Soils
Lipid (ng/g dws % of total)
PC
PE
PG
MMPE
DMPE
Lipid species (total acyl carbons: total double bonds)

Fig. 5.

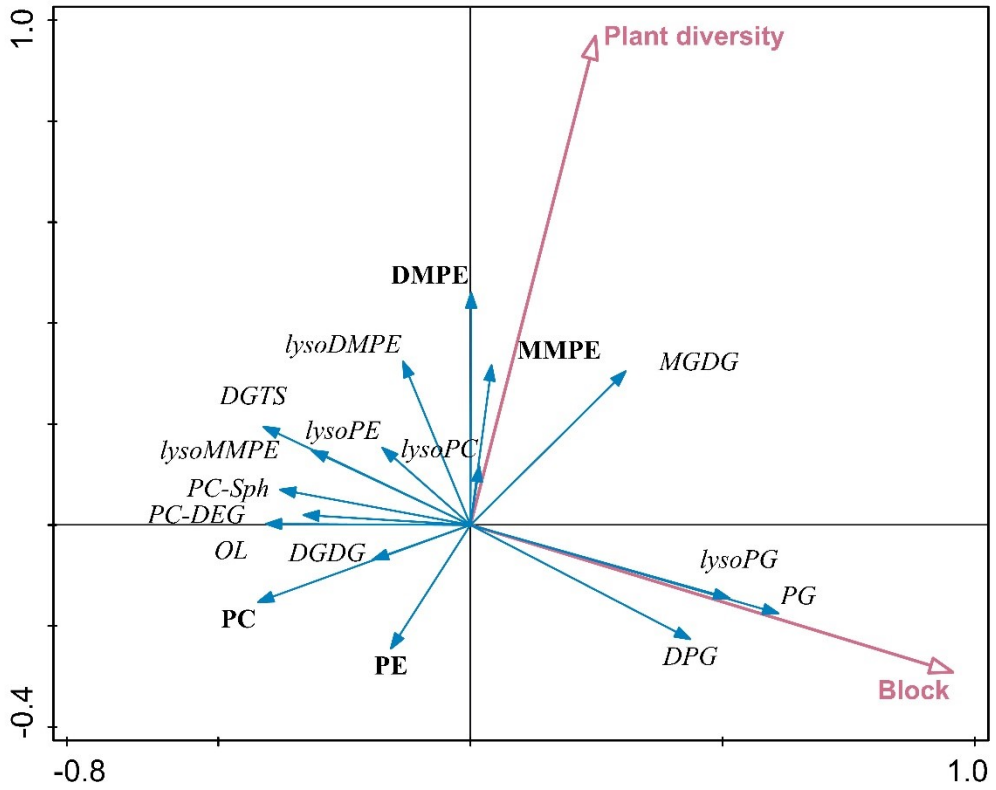

1.0
-0.4
-0.8
1.0
Plant diversity
DMPE
MMPE
MGDG
lysoDMPE
DGTS
lysoPE
lysoPC
lysoMMPE
PC-Sph
PC-DEG
OL
DGDG
PC
PE
lysoPG
PG
DPG
Block

Table 1

Concentration of total polar lipids and relative abundance of intact polar lipid classes in surface soils, plant roots, fungi, Collembola and amoebae

	Soils	Plant roots	Fungi	Collembola	Amoebae
Total polar lipids	μg g^{-1} dws n = 76	mg g^{-1} n = 6	mg g^{-1} n = 11	mg g^{-1} n = 6	mg g^{-1} n = 1
Lipid amount (means ± SD)	43.3 ± 31.9	65.7 ± 44.6	13.9 ± 6.6	1.6 ± 0.9	609.8⋆
Relative abundance of lipid class (%)					
PC	28.3 ± 3.0	12.3 ± 2.7	23.5 ± 7.1	39.2 ± 12.4	26.6
PE	14.0 ± 1.7	11.7 ± 3.1	39.3 ± 3.9	54.1 ± 14.5	31.2
PG	6.2 ± 3.8	7.1 ± 6.6	0.9 ± 2.6	n.d.	12.2
PI	4.0 ± 1.0	1.0 ± 0.5	1.2 ± 1.0	n.d.	2.0
MMPE	3.7 ± 0.5	0.2 ± 0.2	1.2 ± 1.6	n.d.	2.3
DMPE	0.5 ± 0.1	n.d.	n.d.	n.d.	n.d.
MGDG	16.5 ± 3.4	44.3 ± 13.0	n.d.	n.d.	n.d.
DGDG	0.8 ± 0.3	5.9 ± 3.0	n.d.	n.d.	n.d.
DGTS	6.8 ± 1.2	3.2 ± 1.5	8.6 ± 9.4	n.d.	n.d.
OL	9.2 ± 3.4	n.d.	n.d.	n.d.	n.d.
LysoPC	3.8 ± 1.1	7.2 ± 1.6	11.8 ± 5.6	6.7 ± 3.1	15.6
LysoPE	2.6 ± 0.8	6.8 ± 1.4	13.4 ± 5.4	n.d.	10.0
LysoPG	0.2 ± 0.1	0.2 ± 0.1	n.d.	n.d.	n.d.
LysoMMPE	1.0 ± 0.4	0.1 ± 0.1	0.1 ± 0.2	n.d.	n.d.
Others	2.5 ± 1.5	n.d.	n.d.	n.d.	n.d.

n.d., not detected.

⋆, the major properties of the lipid distribution are probably valid (Korn and Wright, 1973; Allen et al., 1974).

Table 2

Lipid molecular species (total numbers of carbons:total double bonds)	Two acyl chains
PC (32:1)	n.d.
PC (33:1)	15:0/18:1
PC (33:2)	15:1/18:1
PC (34:0)	17:0/17:0 > 16:0/18:0
PC (34:1)	n.d.
PC (34:2)	16:1/18:1
PC (35:1)	16:1/19:0
PC (35:2)	16:1/19:1
PC (36:1)	18:0/18:1
PC (36:2)	18:1/18:1
PC (37:2)	18:1/19:1
PC (38:2)	n.d.
PE (30:0)	15:0/15:0 + 14:0/16:0
PE (30:1)	15:1/15:0 + 14:0/16:1
PE (31:1)	15:0/16:1
PE (32:0)	16:0/16:0 > 15:0/17:0
PE (32:1)	16:1/16:0 > 15:0/17:1
PE (32:2)	16:1/16:1
PE (33:0)	16:0/17:0 > 15:0/18:0
PE (33:1)	16:0/17:1 > 16:1/17:1
PE (33:2)	16:1/17:1 > 15:1/18:1
PE (34:0)	17:0/17:0 > 16:0/18:0
PE (34:1)	17:1/17:0 > 16:1/18:0
PE (34:2)	16:1/18:1 > 17:1/17:1
MMPE (31:1)	15:0/16:1 > 14:0/17:1
MMPE (32:1)	16:1/16:0
MMPE (32:2)	16:1/16:1
MMPE (33:1)	16:0/17:1 > 16:1/17:0

MMPE (33:2)	16:1/17:1 > 15:1/18:1
MMPE (34:1)	16:1/18:0 > 17:0/17:1
MMPE (34:2)	16:1/18:1 > 17:1/17:1
MMPE (35:1)	16:1/19:0 > 17:1/18:0
MMPE (35:2)	16:1/19:1 > 17:1/18:1
MMPE (36:2)	18:1/18:1
MMPE (37:2)	18:1/19:1
MMPE (38:2)	n.d.

The acyl groups of the phospholipid classes PC, PE and MMPE from soil samples were identified as acyl anions from their negative ion precursors. The less-than and greater-than characters indicated there were two possible combinations of acyl species in the specific lipid and their intensities were not equal.

Characterization of intact polar lipids in soils for assessing their origin

Supplementary Materials

Table S1. List of investigated organisms

Organisms	classification	number of replicates	sample site
Polysphodylium pallidum	Amoebae	1	-
Folsomia candida	Collembola	3	-
Heteromurus nitidus	Collembola	3	-
Schizophyllum commune	Fungi	3	-
Tricholoma vaccinum	Fungi	3	-
Pisolithus tinctorius	Fungi	3	-
Mucor plumbeus	Fungi	2	-
mixed small herbs	Plant root	1	RB4A06
mixed grass	Plant root	1	RB3A09
mixed tall herbs	Plant root	1	RB1A11
mixed roots of all	Plant root	1	RB3A14
mixed roots of all	Plant root	1	RB1A22
mixed legume	Plant root	1	RB1A12

Details of plant taxonomic information could be found in Roscher et al. (2004).

Fig. S1. Density map plot showing whole range of intact polar lipids detected in a soil sample (B1A21) of the Jena Experiment.

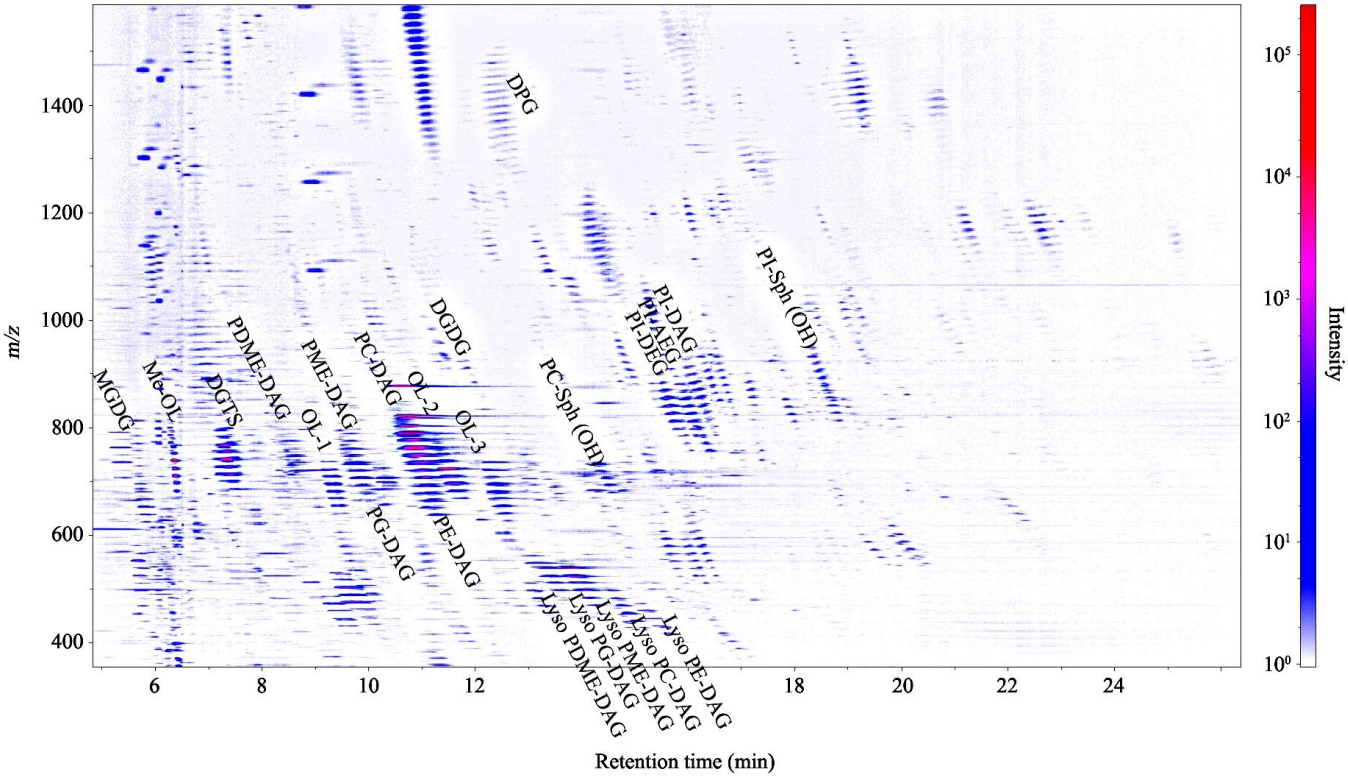


Fig. S2. Molecular species of lysophospholipid classes (weight% of total intact polar lipids analyzed) in the soils (n = 76), plant roots (n = 6), fungi (n = 11) and Collembola (n = 6). The data averaged among different species/samples and the n represents species/samples used for the lipid measurement and data analysis. Values are means ± SD. Individual molecular species in each lipid class was shown in the type of total acyl carbons:total double bonds. Only the most abundant molecular species are shown here.

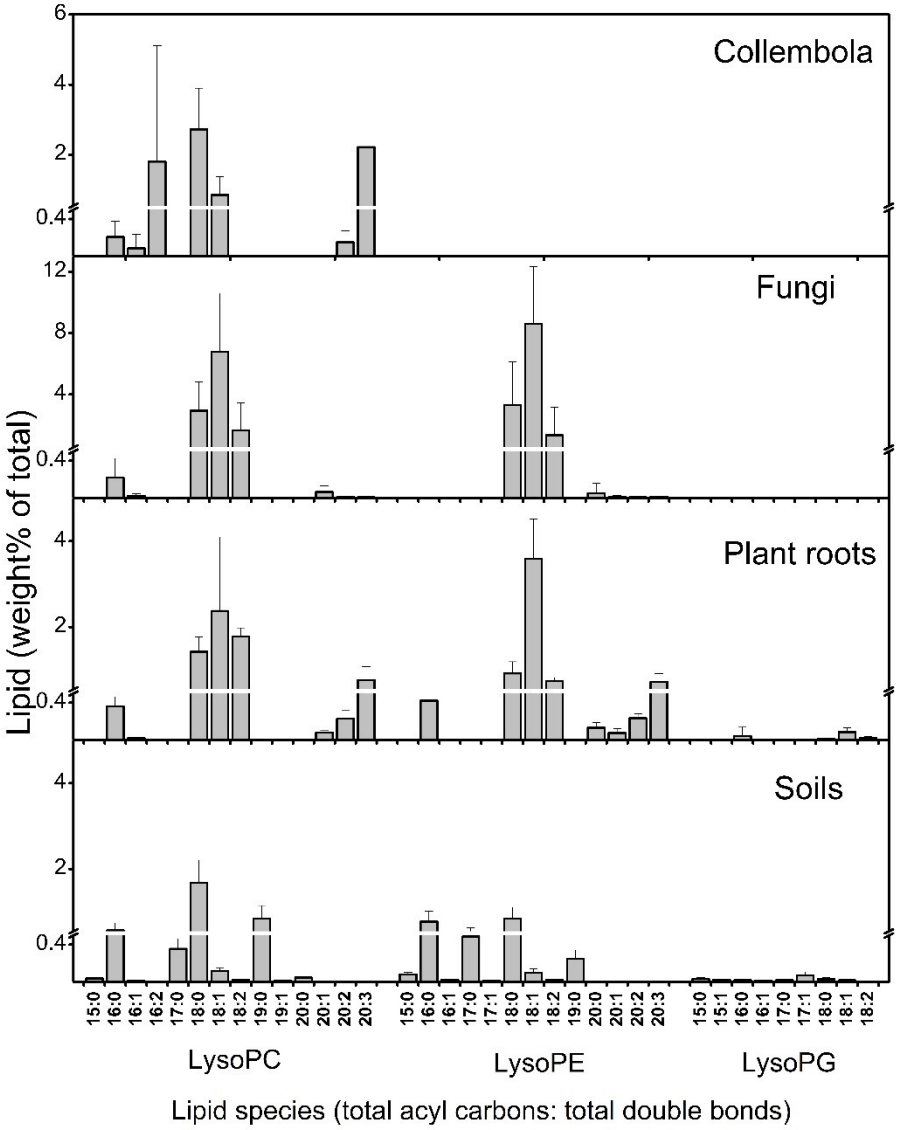
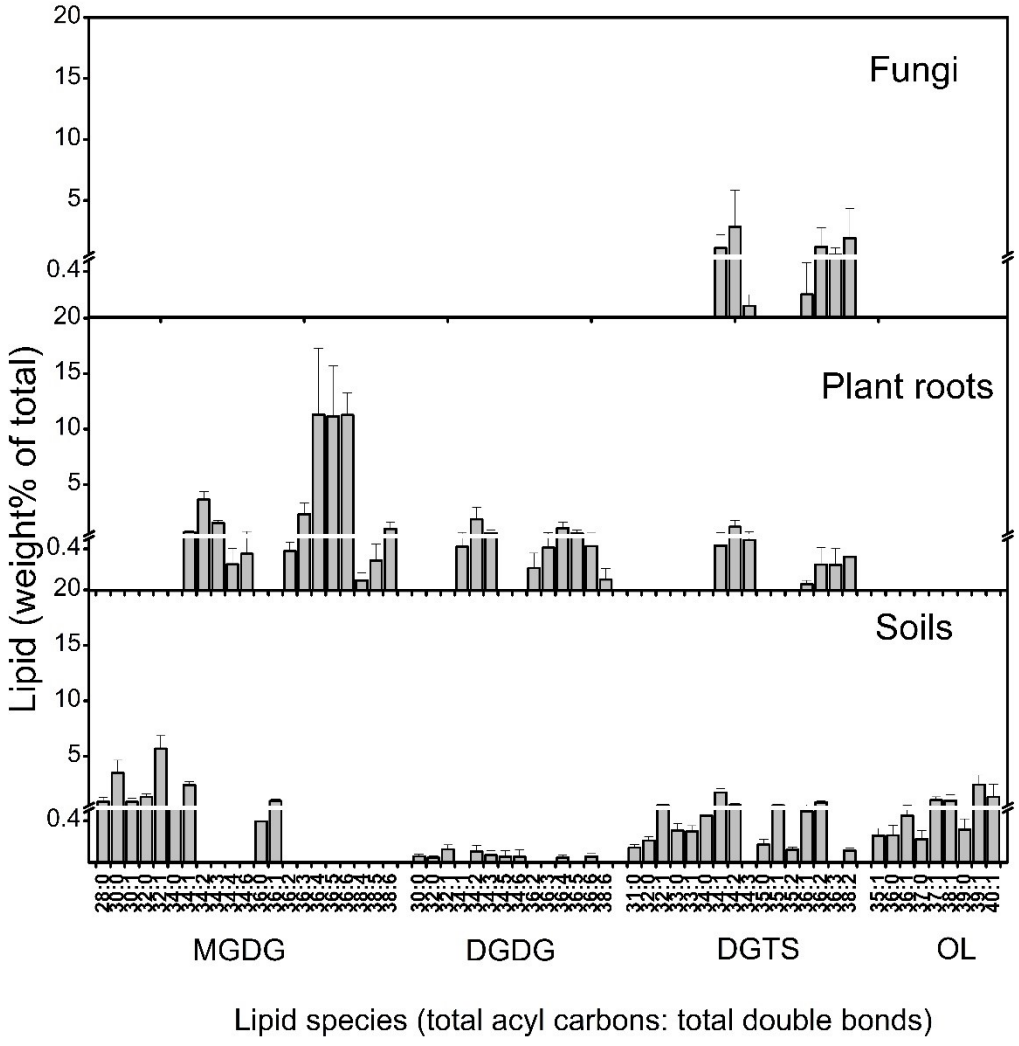


Fig. S3. Molecular species of glycolipid and aminolipid classes (weight% of total intact polar lipids analyzed) in the soils (n = 76), plant roots (n = 6), fungi (n = 11). These lipids were not detected in Collembola. The data averaged among different species/samples and the n represents species/samples used for the lipid measurement and data analysis. Values are means ± SD. Individual molecular species in each lipid class was shown in the type of total acyl carbons:total double bonds. Only the most abundant molecular species are shown here.



5. Discussion and outlook

At the current stage, the advancing analytical technology of lipidomics has greatly driven the field of biogeochemistry and ecology. It is not only useful for studying microbial signal processing, but also meaningful for understanding climate changes, carbon sources and organism interactions.

Constraining the uncertainties of brGDGT derived proxies

The relative distribution of brGDGT membrane lipids in soils can be used to reconstruct mean annual air temperature and soil pH in paleoclimate studies (Weijers et al., 2007). A series of new found isomers of brGDGTs were identified by a modified HPLC separation combined with fraction collection and ether cleavage (Chapter 2). Newly found isomers showed high correlation with sediment pH. This finding may give important implications about the hypothesis of the proxy established on brGDGTs. When pH in the outer membrane changed, which mechanism brGDGT producers choosed to adjust their membrane fluidity to the environmetal changes? The number of cyclization or the position of branched methyl groups? CBT index based on cyclization of brGDGTs should be retested because of newly discovered pentamethylated brGDGTs overlaped with the ones been used for the index. Therefore, our study provide new insights to these membrane lipids and further study is still needed to learn the mechanisms brGDGT producers applied.

Sources and fate of intact polar and core GDGTs in groundwater

In terrestrial subsurface, the response of groundwater to climate changes is of increasing concern. In particular, understanding microbial community sources is fundamental to illuminate the role of microorganisms in carbon cycle and to preserve groundwater (Schwab et al., 2016). In deep critical zone, there are limited carbon and energy sources could support heterotrophic, autotrophic, or methanotrophic microorganisms (Griebler and Lueders, 2009). Thus, the mechanisms underlying microorganisms survive in such extreme environment and the role they act in the carbon cycle are not fully understood, especially

for pristine groundwater (Akob and Küsel, 2011; Jørgensen and Marshall, 2016). In groundwater, organic carbon is a complex and heterogeneous mixture, and its age may reflect the different stage of potential carbon sources for the organisms involved (Nowak et al., 2017; Schwab et al., 2017). Heterotrophs may use modern organic carbon from surface soils, or fossil organic carbon from sedimentary rocks (Biddle et al., 2006). Carbon sources for chemolithoautotrophic microorganisms that may serve also as terminal electron acceptor for dissimilatory CO_2-reducing microorganisms such as methanogens and acetogens (Hug et al., 2015; Smith et al., 2012; Zinger et al., 2012). The biomarker lipids from these microorganisms may thus reflect their carbon sources and the metabolic pathway (e.g., heterotrophy, autotrophy, methanotrophy).

Groundwater ecosystems possess complex habitats for diverse microbial commnunities (Bethke et al., 2008; Griebler and Lueders, 2009). Can we track the biological signal of surface conditions downward in groundwater? How these signals affect the diversity and function of the subsurface residence? This is the first time lipid biomarker GDGTs was applied in terrestrial subsurface (Chapter 3). Both living and dead bacterial and archaea GDGT producers could help understand this extreme environment. For them, a slight environmental fluctuation such as the runoff from the surface soils is more likely to be an 'extreme' challenge. Thus, GDGTs are ideal tools for tracing sources and signal transportation.

Composition of intact polar membrane lipids in soils and their environmental factors

Plant diversity is a significant driver of soil organic matter dynamics (Prober et al., 2015). Soil microbial communities have been shown to regulate several plant-soil interactions, such as soil carbon cycling, decomposition and storage (Schimel and Schaeffer, 2012). In recent studies the positive effect of plant diversity on soil organic carbon storage has been reported (Lange et al., 2015), however, the mechanisms of how plant diversity influence soil microbial distribution and function are still largely unknown. Hence the lipid markers may offer great information about microbial taxonomic specificity in soils, how microorganisms distribute, behave and interact with plants.

Nearly all intact polar membrane lipid anlysis of microbial commnunities were carried out in cultures in the laboratory (Geiger et al., 2013; Sohlenkamp and Geiger, 2016). In the real environments, for example in soils, scientists assessed microbial commnunity by analyzing phospholipid fatty acids (Frostegård et al., 1993; Zelles, 1997). Changes in the community structures then were interpreted based on the database of pure cultures (Zelles, 1999). There's a gap between cultured lipids and lipids in natures. Are their composition belong to a single species still similar? Is there specific membrane lipid marker for unique commnuity? How they distributed in surface soils and what are their sources? For these reasons, we provided a full lipid distribution in soils. The odd number chain length of acyl species and low saturation of soil membrane lipids suggested they were mainly derived from bacteria (Zelles, 1997, 1999). To be noticed, plant diversity affected the methylation biosynthesis pathway for PC formation in soil bacteria. This pathway could only be found in some pathogens and nitrogen fixing bacteria (López-Lara et al., 2003; Martínez-Morales et al., 2003; Sohlenkamp et al., 2003). Does plant diversity control the production of nitrogen fixing bacteria? For this, we measured 16s rRNA gene in the same soil samples and evaluted the correlation of plant diversity and microbial commnunities. Meanwhile we also calculated the relationship between δ^{15}N isotopic signal and the relative abundance of nitrogen fixers (Fig. 7.1).

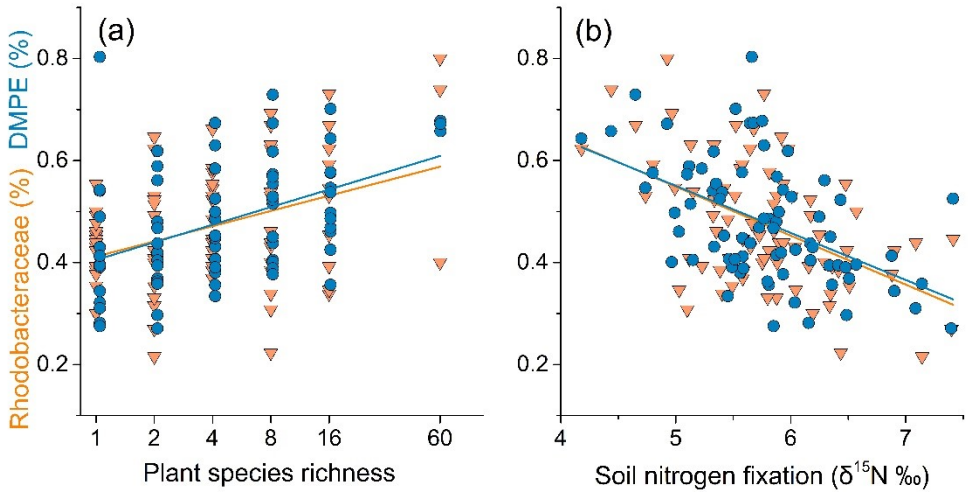


Fig. 7.1. Linear correlation of (left) Plant diversity, (right) δ^{15}N isotope with relative abundance of *Rhodobacter* and membrane lipid DMPE.

Both plant diversity and $\delta^{15}N$ isotope showed significant correlation with a pivotal organism – *Rhodobacter*. In addition, membrane lipid DMPE showed similar trend as *Rhodobacter* community, suggesting these lipids were mainly produced by them. Many nitrogen-fixing fixers work only in anaerobic conditions because the nitrogenase enzyme is highly sensitive to oxygen (Kahindi et al., 1997; Zahran, 1999). In the symbiotic nitrogen-fixers such as *Rhizobium*, the root nodules contain oxygen-preventing molecules such as leghaemoglobin, which allows *Rhizobium* to fix nitrogen in the oxic conditions (Fischer, 1994; Gage, 2004; Salvagiotti et al., 2008). While *Rhodobacter* has an intricate system for sensing O_2 tensions, called chromatophores. The photosynthetic and nitrogen fixation work are housed in these invaginations (Madigan and Jung, 2009; Masepohl et al., 2005; Tai et al., 1986). It is generally believed that associated nitrogen fixing rates are greater than free-living ones in ecosystems (Vadakattu and Paterson, 2006; Zhan and Sun, 2012). However, we couldn't find any correlation between $\delta^{15}N$ isotope and associated nitrogen fixers. Thus, the free-living photosynthetic bacteria *Rhodobacter* may be play dominant role on nitrogen fixation in grassland and their abundance is affected by the plant diversity. Future work should be tested on the role of *Rhodobacter* in the biological nitrogen fixation and their biomarkers.

6. Summary

Microorganisms have the ability to control the biophysical properties of their membrane lipids to thrive in natural environments (Zhang and Rock, 2008). Different microbial species possess different membrane lipid compositions, even the composition of a single species is not always constant, but depends on the habitat conditions (Sohlenkamp and Geiger, 2016). Microorganisms adjust their membrane lipid composition by modifying the types of glycerol backbone (chain length, saturation, and branching) and head groups (Schouten et al., 2013; Strahl and Errington, 2017). Therefore, signatures of membrane lipids in the environmental samples could be used as biomarkers reflecting the physicochemical conditions and the community structures (Brocks and Banfield, 2009; Brocks and Pearson, 2005; Castañeda and Schouten, 2011). With the increasing knowledge of lipid structures and their properties, the interpretation of lipid-based biomarker in environmental samples would be more accurate. By using lipidomics, this thesis aimed at (*i*) identifying new lipid isomers to help improve the precision of its derived proxy, (*ii*) expanding the application of lipid biomarkers to the terrestrial subsurface critical zone for tracing the signal of surface down, (*iii*) providing a full lipid profile in the soils, investigating their sources and potential possibility as novel chemotaxonomic markers for bacteria.

The recently discovered 6-methyl brGDGTs improved the accuracy of the derived biomarkers for mean annual air temperature (MBT) and soil pH (CBT and IBT). However, the errors of the proxies were still substantial (ca. 3°C for MBT). In the first manuscript (Chapter 4), we described a new HPLC/MS protocol allowing the isolation of new brGDGT isomers that co-eluted with the previous known 5- and 6-mthyl brGDGTs. In this study, we successfully identified their structures from a mixture of 102 lake sediments. The novel identified 7-methyl brGDGT isomers accounted for 6% of the total brGDGTs in Chinese and Cameroon lake sediments. One isomer named IIa_7 showed highest correlation

with sediment pH. In addition, two novel pentamethylated brGDGTs were also tentatively identified based on the mass spectra of their ether-cleaved hydrocarbons. Thus, a recalibration of the proxies within these new isomers and the understanding of the mechanism upon which bacteria produce different brGDGT isomers are needed in the future studies.

In Chapter 5, we investigated the microbial communities in groundwater (Hainich Critical Zone Exploratory) by analyzing membrane lipid br- and isoGDGTs. GDGTs from dead and living organisms were determined by the form of core and intact polar lipid, respectively. We estimated the source of GDGTs in groundwater by comparing their distribution to the ones from soils of potential recharge areas. We found both bacterial and archaeal GDGTs were mainly produced in situ in groundwater samples. In the living pool of soils, brGDGTs were less abundant than those of isoGDGTs, while in the dead pool, the situation was opposite. This suggested brGDGT producing bacteria were more active and regenerated faster than isoGDGT-producing archaea in soils. A similar trend was also found in groundwater that indicated higher activity (i.e., cell division) of bacteria than of archaea. Moreover, the 16S rRNA archaeal gene data supported indigenous production of archaea-derived isoGDGTs in groundwater, mainly from Marine Group I.1a Thaumarchaeota. This first GDGT study in groundwater demonstrated that harsh subsurface environments host an in situ bacterial and archaeal community.

In Chapter 6, we provide a lipidomics containing over 300 membrane lipids in the surface soils of a grassland. The composition, metabolism and function of membrane lipids are well studied in pure cultures. But there's lack of knowledge of their distribution, sources and function in natural environments. We showed the distribution of intact polar membrane lipids in 76 soil samples differing in the plant community and soil texture. In order to determine the source of soil lipid profile we compared them to those of plant roots, fungi, collembola as well as amoebae. The soil lipid profile showed more diverse and evenly distributed than other potential producers. Soil membrane lipids contain a distinctively large amount of odd number carbon chain acyl species and low levels of unsaturation, which could not be found in plant roots and other organisms. This indicated they were mainly coming from bacteria rather than plants and fungi. their communities

and compositions are more complex in natural environments than in cultured conditions. Compare to PLFA, lipidomics may provide more information about the function and interaction of different lipid head groups. As the distribution of these bacterial membrane lipids are not fully studied in the real natures, more researches on the environmental samples are needed to help understand their function, composition and drivers.

In this thesis, we show lipidomics is still a useful and powerful technology to study microbial composition, function, metabolic pathway as well as interaction with other organisms in the environments. Novel approaches for analysis of the entire microbial lipidome are still warranted. A fundamental goal of microbial lipid biomarkers is to understand major role of microorganisms in the global carbon cycle. Environmental factors or microbial community structures, which is more import for lipid distribution in environmtal samples? As the technologies of lipidomics advance, future work of microorganisms in the environments should be focus on similtaneous characters of both lipidomics and genes. With this comprehensive information, the evolutionary and taxonomic roles of microorganism can be more deeply demonstrated.

Zusammenfassung

Mikroorganismen sind in der Lage die biophysikalischen Eigenschaften ihrer Membranlipide anzupassen um in ihrer natürlichen Umgebung erfolgreich zu gedeihen (Zhang and Rock, 2008). Die Zusammensetzung der Membranlipide unterscheidet sich zwischen verschiedenen Mikroorganismen und ist selbst bei derselben Spezies nicht immer konstant, sondern variiert in Abhängigkeit des Lebensraumes (Sohlenkamp and Geiger, 2016). Mikroorganismen passen ihre Membranlipidzusammensetzung durch eine Veränderung der Glycerolgerüste (Kettenlänge, Sättigung und Verzweigung) sowie der Kopfgruppen an (Schouten et al., 2013; Strahl and Errington, 2017). Aufgrund dieser molekularen Struktursignaturen können Membranlipide als Biomarker verwendet werden und repräsentieren dabei sowohl die physikochemischen Bedingungen des jeweiligen Lebensraumes als auch die Zusammensetzung der mikrobiellen Gemeinschaft (Brocks and Banfield, 2009; Brocks and Pearson, 2005; Castañeda and Schouten, 2011). Daher ist eine Steigerung des Wissensstandes über Lipidstrukturen und ihrer Eigenschaften essenziell um genauere Interpretationen von lipidbasierten Biomarkern zu ermöglichen. Die Ziele der vorliegenden Doktorarbeit waren (i) die Identifikation neuer Lipidisomere um die Präzision der abgeleiteten Indikatoren zu erhöhen, (ii) die Erweiterung der Anwendungsgebiete von Lipidbiomarkern auf natürliche Proben aus der Kritischen Zone um terrestrische Oberflächensignale in die Untergrundschichten zu verfolgen und (iii) die Erstellung eines vollständigen Lipidprofiles von Böden mit speziellem Fokus auf die möglichen individuellen Quellen der Lipide und ihr Potential als neuartige chemotaxonomische Marker für Bakterien.

Die kürzlich entdeckten 6-methyl brGDGTs verbesserten die Genauigkeit der von ihnen abgeleiteten Biomarker für Jahresdurchschnittstemperaturen und pH-Werte im Boden. Jedoch waren die resultierenden Fehlerbereiche mit ca. 3 °C für die Jahresdurchschnittstemperatur weiterhin erheblich. Im ersten Manuskript (Kapitel 4)

beschrieben wir ein neues HPLC/MS Protokoll, das es erlaubt neue brGDGT Isomere zu isolieren, die mit den bekannten 5- und 6-methyl brGDGTs koeluieren. Die so identifizierten neuen 7-methyl brGDGT Isomere machten 6% der gesamten brGDGTs in Proben von Seesedimenten aus China und Kamerun aus. Das Isomer IIa_7 zeigte die stärkste Korrelation mit dem pH-Wert der Sedimente. Weiterhin konnten auf Basis der Massenspektren von Kohlenwasserstoffen, die an der Etherbindung abgespalten wurden, zwei neue pentamethylierte brGDGTs vorläufig identifiziert werden. In zukünftigen Studien sollte daher eine Rekalibration der existierenden Indikatoren mit diesen neuen Isomeren durchgeführt werden und besonderer Fokus auf die Mechanismen gelegt werden mit denen Bakterien unterschiedliche brGDGT Isomere produzieren.

In Kapitel 5 untersuchten wir mikrobielle Gemeinschaften im Grundwasser (Hainich Kritische Zone Exploratorium) anhand ihrer br- und isoGDGT Membranlipidprofile. GDGTs von lebendigen Organismen wurden anhand von intakten polaren Lipiden, GDGTs von toten Organismen anhand von Lipidskeletten bestimmt. Die Quelle der GDGTs im Grundwasser wurde nach ihrer Verteilung im Vergleich zu Bodenproben aus den potentiellen Grundwasserneubildungsflächen eingeschätzt. Wir konnten zeigen, dass GDGTs von Bakterien und Archaeen hauptsächlich in-situ im Grundwasser gebildet wurden. Bei der Bodenprobenanalyse im Bezug auf die lebendigen Organismen waren brGDGTs im Vergleich zu isoGDGTs abgereichert. Für die bereits abgestorbenen Organismen zeigte sich eine gegensätzliche Verteilung. Dies deutet darauf hin, dass in den Böden die brGDGT produzierenden Bakterien aktiver waren und sich schneller regeneriert haben als die isoGDGT produzierenden Archaeen. Im Grundwasser zeichnete sich ein vergleichbarer Trend ab, der ebenso auf eine höhere Aktivität (Zellteilung) von Bakterien im Vergleich zu Archaeen hindeutete. Genetische Daten aus 16S rRNA Analysen von Archaeen unterstützten die Schlussfolgerung der in-situ Bildung von isoGDGTs und deuteten auf die Marine Gruppe I.1a Thaumarchaeota als hauptsächlichen Poduzenten hin. Diese erste GDGT-Studie im Grundwasser zeigte, dass auch raue unterirdische Umgebungen in-situ eine Gemeinschaft von Bakterien und Archaeen beherbergen.

In Kapitel 6 stellen wir lipidomische Analysen von Graslandböden vor, die über 300 Membranlipide umfassen. Es existiert ein breiter Wissensschatz über die

Zusammensetzung, Metabolismus und Funktion von Membranlipiden in Reinkulturen. Jedoch sind in natürlichen Umgebungen ihre Verteilung, Quellen und Funktionen nur unzureichend bekannt. In unserer Studie zeigten wir die Verteilung von intakten polaren Membranlipiden aus 76 Bodenproben, die sich in der Zusammensetzung der lokal wachsenden Pflanzengesellschaft und der Bodentextur unterschieden. Um ihre Quellen zu bestimmen verglichen wir die Lipidprofile der Böden zu denen von Wurzeln, Pilzen sowie Collembola und Amoebae Spezies. Die Lipidprofile der Böden waren diverser und gleichmäßiger verteilt als die der potenziellen Produzenten. Die Membranlipide in den Böden enthielten große Mengen von Acylspezies mit ungerader Kettenlänge, die nur wenig ungesättigt waren und in Pflanzenwurzeln sowie anderen Organismen nicht auftraten. Dies deutete darauf hin, dass sie eher von Bakterien stammten als von Pflanzen oder Pilzen. Die Zusammensetzung von Membranlipiden in der Natur ist deutlich komplexer als unter Kulturbedingungen und im Vergleich zu PLFA-Analysen kann durch Lipidomics mehr Information über die Funktion und Interaktion verschiedener Lipid-Kopfgruppen erhalten werden. Da die Verteilung der Membranlipide von Bakterien in der Natur jedoch noch nicht vollständig geklärt ist, ist weitere Forschung nötig um ihre Funktion in der Umwelt zu verstehen.

Ein grundlegendes Ziel beim Einsatz von Lipidbiomarkern ist es die Rolle von Mikroorganismen im globalen Kohlenstoffkreislauf zu verstehen. Sind Umweltfaktoren oder die Zusammensetzung der mikrobiellen Gemeinschaft wichtiger für die Lipidverteilungen in natürlichen Proben? Zukünftige wissenschaftliche Arbeit im Bereich der Lipidbiomarker wird einen parallelen Fokus sowohl auf die Charakterisierung von Membranlipiden als auch auf genetische Analysen benötigen. Die durch diesen Ansatz zu erhaltenden ausführlichen Informationen werden entscheidend sein um die Rolle von Membranlipiden in einem evolutionären und taxonomischen Kontext umfassend darzulegen.

References

Akob, D.M., Küsel, K., 2011. Where microorganisms meet rocks in the Earth's Critical Zone. Biogeosciences 8, 3531-3543.

Alongi, D.M., 1994. The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems, Ecology and conservation of southeast Asian marine and freshwater environments including wetlands. Hydrobiologia 285, 19-32.

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems, invertebrate biodiversity as bioindicators of sustainable landscapes. Agriculture, Ecosystems and Environment 74, 19–31.

Amann, R.I., Ludwig, W., Schleifer, K.H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews 59, 143-169.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10, 215-221.

Arondel, V., Benning, C., Somerville, C.R., 1993. Isolation and functional expression in Escherichia coli of a gene encoding phosphatidylethanolamine methyltransferase (EC 2.1.1.17) from *Rhodobacter sphaeroides*. Journal of Biological Chemistry 268, 16002-16008.

Auer, J., Lechner, K., Bock, A., 1989. Gene organization and structure of two transcriptional units from *Methanococcus* coding for ribosomal proteins and elongation factors. Canadian journal of microbiology 35, 200-204.

Auguet, J.C., Casamayor, E.O., 2008. A hotspot for cold *crenarchaeota* in the neuston of high mountain lakes. Environmental Microbiology 10, 1080-1086.

Aygun-Sunar, S., Mandaci, S., Koch, H.G., Murray Ian, V.J., Goldfine, H., Daldal, F., 2006. Ornithine lipid is required for optimal steady-state amounts of c-type cytochromes in *Rhodobacter capsulatus*. Molecular Microbiology 61, 418-435.

Azam, F., Fenchel, T., Field, J.G., Gray, J., Meyer-Reil, L., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Marine ecology progress series, 257-263.

Babiuk, L., Paul, E., 1970. The use of fluorescein isothiocyanate in the determination of the bacterial biomass of grassland soil. Canadian Journal of Microbiology 16, 57-62.

Barns, S.M., Cain, E.C., Sommerville, L., Kuske, C.R., 2007. Acidobacteria phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum. Applied and environmental microbiology 73, 3113-3116.

Bechtel, A., Smittenberg, R.H., Bernasconi, S.M., Schubert, C.J., 2010. Distribution of branched and isoprenoid tetraether lipids in an oligotrophic and a eutrophic Swiss lake: Insights into sources and GDGT-based proxies. Organic Geochemistry 41, 822-832.

Benning, C., Huang, Z.H., Gage, D.A., 1995. Accumulation of a novel glycolipid and a betaine lipid in cells of *Rhodobacter sphaeroides* grown under phosphate limitation. Archives of Biochemistry and Biophysics 317, 103-111.

Berg, G., 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology 84, 11-18.

Bethke, C.M., Ding, D., Jin, Q., Sanford, R.A., 2008. Origin of microbiological zoning in groundwater flows. Geology 36, 739-742.

Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T.J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H., Hinrichs, K.-U., 2006. Heterotrophic *archaea* dominate sedimentary subsurface ecosystems off Peru. Proceedings of the National Academy of Sciences of the United States of America 103, 3846-3851.

Blaga, C.I., Reichart, G.-J., Heiri, O., Damste, J.S.S., 2009. Tetraether membrane lipid distributions in water-column particulate matter and sediments: a study of 47 European lakes along a north-south transect. Journal of paleolimnology 41, 523-540.

Bohlool, B.B., Ladha, J.K., Garrity, D.P., George, T., 1992. Biological nitrogen fixation for sustainable agriculture: A perspective. Plant and Soil 141, 1-11.

Brügger, B., 2014. Lipidomics: Analysis of the lipid composition of cells and subcellular organelles by electrospray ionization mass spectrometry. Annual Review of Biochemistry 83, 79-98.

Brocks, J.J., Banfield, J., 2009. Unravelling ancient microbial history with community proteogenomics and lipid geochemistry. Nature Reviews Microbiology 7, 601-609.

Brocks, J.J., Pearson, A., 2005. Building the biomarker tree of Life. Reviews in Mineralogy and Geochemistry 59, 233-258.

Cañavate, J.P., Armada, I., Ríos, J.L., Hachero-Cruzado, I., 2016. Exploring occurrence and molecular diversity of betaine lipids across taxonomy of marine microalgae. Phytochemistry 124, 68-78.

Castañeda, I.S., Schouten, S., 2011. A review of molecular organic proxies for examining modern and ancient lacustrine environments. Quaternary Science Reviews 30, 2851-2891.

Chan, O.C., Yang, X., Fu, Y., Feng, Z., Sha, L., Casper, P., Zou, X., 2006. 16S rRNA gene analyses of bacterial community structures in the soils of evergreen broad-leaved forests in south-west China. FEMS Microbiology Ecology 58, 247-259.

Conover, G.M., Martinez-Morales, F., Heidtman, M.I., Luo, Z.-Q., Tang, M., Chen, C., Geiger, O., Isberg, R.R., 2008. Phosphatidylcholine synthesis is required for optimal function of Legionella pneumophila virulence determinants. Cellular Microbiology 10, 514-528.

Cronan, J.E., 1978. Molecular biology of bacterial membrane lipids. Annual Review of Biochemistry 47, 163-189.

Cronan, J.E., 2003. Bacterial membrane lipids: Where do we stand? Annual Review of Microbiology 57, 203-224.

Cronan, J.E., Jr., Gelmann, E.P., 1975. Physical properties of membrane lipids: biological relevance and regulation. Bacteriological reviews 39, 232-256.

De Jonge, C., Hopmans, E.C., Stadnitskaia, A., Rijpstra, W.I.C., Hofland, R., Tegelaar, E., Sinninghe Damsté, J.S., 2013. Identification of novel penta- and hexamethylated branched glycerol dialkyl glycerol tetraethers in peat using HPLC–MS2, GC–MS and GC–SMB-MS. Organic Geochemistry 54, 78-82.

De Jonge, C., Hopmans, E.C., Zell, C.I., Kim, J.-H., Schouten, S., Sinninghe Damsté, J.S., 2014. Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: Implications for palaeoclimate reconstruction. Geochimica et Cosmochimica Acta 141, 97-112.

de Rudder, K.E., Thomas-Oates, J.E., Geiger, O., 1997. Rhizobium meliloti mutants deficient in phospholipid N-methyltransferase still contain phosphatidylcholine. Journal of Bacteriology 179, 6921-6928.

De Rudder, K.E.E., López-Lara, I.M., Geiger, O., 2000. Inactivation of the gene for phospholipid N-methyltransferase in Sinorhizobium meliloti: phosphatidylcholine is required for normal growth. Molecular Microbiology 37, 763-772.

DeChavigny, A., Heacock, P.N., Dowhan, W., 1991. Sequence and inactivation of the pss gene of *Escherichia coli*. Phosphatidylethanolamine may not be essential for cell viability. Journal of Biological Chemistry 266, 5323-5332.

Devaiah, S.P., Roth, M.R., Baughman, E., Li, M., Tamura, P., Jeannotte, R., Welti, R., Wang, X., 2006. Quantitative profiling of polar glycerolipid species from organs of wild-type Arabidopsis and a phospholipase Dα1 knockout mutant. Phytochemistry 67, 1907-1924.

Ding, S., Xu, Y., Wang, Y., He, Y., Hou, J., Chen, L., He, J.S., 2015. Distribution of branched glycerol dialkyl glycerol tetraethers in surface soils of the Qinghai–Tibetan Plateau: implications of brGDGTs-based proxies in cold and dry regions. Biogeosciences 12, 3141-3151.

Dowhan, W., 1997. Molecular basis for membrane phospholipid diversity: Why are there so many lipids? Annual Review of Biochemistry 66, 199-232.

Eichenberger, W., 1993. Betaine lipids in lower plant. Distribution of DGTS, DGTA and phospholipids, and the intracellular localization and site of biosynthesis of DGTS. Plant physiology and Biochemistry 31, 213-221.

Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A.C.W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W.W., Scheu, S., 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91, 485-496.

Eisenhauer, N., Reich, P.B., Scheu, S., 2012. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. Basic and Applied Ecology 13, 571-578.

Elling Felix, J., Könneke, M., Nicol Graeme, W., Stieglmeier, M., Bayer, B., Spieck, E., de la Torre José, R., Becker Kevin, W., Thomm, M., Prosser James, I., Herndl Gerhard, J., Schleper, C., Hinrichs, K.U., 2017. Chemotaxonomic characterisation of the thaumarchaeal lipidome. Environmental Microbiology 19, 2681-2700.

Epand, R.M., Epand, R.F., 2009. Lipid domains in bacterial membranes and the action of antimicrobial agents. Biochimica et Biophysica Acta (BBA) - Biomembranes 1788, 289-294.

Epand, R.M., Epand, R.F., 2010. Bacterial membrane lipids in the action of antimicrobial agents. Journal of Peptide Science 17, 298-305.

Fahy, E., Subramaniam, S., Murphy, R.C., Nishijima, M., Raetz, C.R.H., Shimizu, T., Spener, F., van Meer, G., Wakelam, M.J.O., Dennis, E.A., 2009. Update of the lipid maps comprehensive classification system for lipids. Journal of Lipid Research 50, 9-14.

Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The microbial engines that drive earth's biogeochemical cycles. Science 320, 1034-1039.

FAO-Unesco, 1997. Soil map of the world : revised legend with corrections and updates. ISRIC, Wageningen.

Fenchel, T., Ramsing, N., 1992. Identification of sulphate-reducing ectosymbiotic bacteria from anaerobic ciliates using 16S rRNA binding oligonucleotide probes. Archives of microbiology 158, 394-397.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2011. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The Isme Journal 6, 1007-1017.

Fischer, C., Roscher, C., Jensen, B., Eisenhauer, N., Baade, J., Attinger, S., Scheu, S., Weisser, W.W., Schumacher, J., Hildebrandt, A., 2014. How do earthworms, soil texture and plant composition affect infiltration along an experimental plant diversity gradient in grassland? PLOS ONE 9, e98987.

Fischer, H.M., 1994. Genetic regulation of nitrogen fixation in rhizobia. Microbiological Reviews 58, 352-386.

Flynn, T.M., Sanford, R.A., Ryu, H., Bethke, C.M., Levine, A.D., Ashbolt, N.J., Santo Domingo, J.W., 2013. Functional microbial diversity explains groundwater chemistry in a pristine aquifer. BMC microbiology 13, 146.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences of the United States of America 102, 14683-14688.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Frostegård, Å., Bååth, E., Tunlio, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723-730.

Gadd, G.M., 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156, 609-643.

Gage, D.J., 2004. Infection and invasion of roots by symbiotic, nitrogen-fixing *Rhizobia* during nodulation of temperate legumes. Microbiology and Molecular Biology Reviews 68, 280-300.

Geiger, O., González-Silva, N., López-Lara, I.M., Sohlenkamp, C., 2010a. Amino acid-containing membrane lipids in bacteria. Progress in Lipid Research 49, 46-60.

Geiger, O., López-Lara, I.M., Sohlenkamp, C., 2013. Phosphatidylcholine biosynthesis and function in bacteria. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1831, 503-513.

Geiger, O., Sohlenkamp, C., López-Lara, I.M., 2010b. Formation of bacterial membrane lipids: pathways, enzymes, reactions, in: Timmis, K.N. (Ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 395-407.

Geske, T., vom Dorp, K., Dörmann, P., Hölzl, G., 2013. Accumulation of glycolipids and other non-phosphorous lipids in Agrobacterium tumefaciens grown under phosphate deprivation. Glycobiology 23, 69-80.

Gill, C., 1975. Effect of growth temperature on the lipids of Pseudomonas fluorescens. Microbiology 89, 293-298.

Gliozzi, A., Paoli, G., De Rosa, M., Gambacorta, A., 1983. Effect of isoprenoid cyclization on the transition temperature of lipids in thermophilic archaebacteria. Biochimica et Biophysica Acta (BBA)-Biomembranes 735, 234-242.

Green, C.T., Scow, K.M., 2000. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeology Journal 8, 126-141.

Griebler, C., Lueders, T., 2009. Microbial biodiversity in groundwater ecosystems. Freshwater Biology 54, 649-677.

Hölzl, G., Dörmann, P., 2007. Structure and function of glycoglycerolipids in plants and bacteria. Progress in Lipid Research 46, 225-243.

Hamilton, T.L., Bryant, D.A., Macalady, J.L., 2016. The role of biology in planetary evolution: cyanobacterial primary production in low-oxygen Proterozoic oceans. Environmental microbiology 18, 325-340.

Han, X., Gross, R.W., 2003. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. Journal of Lipid Research 44, 1071-1079.

Han, X., Gross, R.W., 2005. Shotgun lipidomics: Electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. Mass Spectrometry Reviews 24, 367-412.

Han, X., Yang, K., Gross, R.W., 2012. Multi-dimensional mass spectrometry-based shotgun lipidomics and novel strategies for lipidomic analyses. Mass Spectrometry Reviews 31, 134-178.

Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J., Goodman, R.M., 1998. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chemistry & biology 5, 245-249.

Harvey, H.R., Fallon, R.D., Patton, J.S., 1986. The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. Geochimica et Cosmochimica Acta 50, 795-804.

He, J.-z., Shen, J.-p., Zhang, L.-m., Zhu, Y.-g., Zheng, Y.-m., Xu, M.-g., Di, H., 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environmental Microbiology 9, 2364-2374.

Hopmans, E.C., Weijers, J.W., Schefuß, E., Herfort, L., Sinninghe Damsté, J.S., Schouten, S., 2004. A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. Earth and Planetary Science Letters 224, 107-116.

Horton, K., Norton, J., Horwath, W., Paul, E., 1987. Determination of soil microbial biomass C using the chloroform fumigation incubation method. Bull. Ecol. Soc. Am.;(United States) 68, 459- 467.

Hug, L.A., Thomas, B.C., Brown, C.T., Frischkorn, K.R., Williams, K.H., Tringe, S.G., Banfield, J.F., 2015. Aquifer environment selects for microbial species cohorts in sediment and groundwater. The ISME journal 9, 1846-1856.

Jørgensen, B.B., Marshall, I.P.G., 2016. Slow microbial life in the seabed. Annual review of marine science 8, 311-332.

Janssen, P.H., Yates, P.S., Grinton, B.E., Taylor, P.M., Sait, M., 2002. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. Applied and environmental microbiology 68, 2391-2396.

Jia, Z., Conrad, R., 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environmental Microbiology 11, 1658-1671.

Kaeberlein, T., Lewis, K., Epstein, S.S., 2002. Isolating" uncultivable" microorganisms in pure culture in a simulated natural environment. Science 296, 1127-1129.

Kahindi, J.H.P., Woomer, P., George, T., de Souza Moreira, F.M., Karanja, N.K., Giller, K.E., 1997. Agricultural intensification, soil biodiversity and ecosystem function in the tropics: the role of nitrogen-fixing bacteria. Applied Soil Ecology 6, 55-76.

Kate, M., 1993. Chapter 9 Membrane lipids of archaea, in: Kates, M., Kushner, D.J., Matheson, A.T. (Eds.), New Comprehensive Biochemistry. Elsevier, pp. 261-295.

Kennedy, E.P., Weiss, S.B., 1956. The function of cytidine coenzymes in the biosynthesis of phospholipides. Journal of Biological Chemistry 222, 193-214.

Kent, C., 1995. Eukaryotic phospholipid biosynthesis. Annual review of biochemistry 64, 315-343.

Kim, J.-H., Schouten, S., Hopmans, E.C., Donner, B., Sinninghe Damsté, J.S., 2008. Global sediment core-top calibration of the TEX86 paleothermometer in the ocean. Geochimica et Cosmochimica Acta 72, 1154-1173.

Kimura, M., Arndt, E., Hatakeyama, T., Hatakeyama, T., Kimura, J., 1989. Ribosomal proteins in halobacteria. Canadian Journal of Microbiology 35, 195-199.

Kodaki, T., Yamashita, S., 1987. Yeast phosphatidylethanolamine methylation pathway. Cloning and characterization of two distinct methyltransferase genes. Journal of Biological Chemistry 262, 15428-15435.

Koga, Y., Morii, H., 2005. Recent advances in structural research on ether lipids from archaea including comparative and physiological aspects. Bioscience, biotechnology, and biochemistry 69, 2019-2034.

Krishnamoorthy, G., Wolloscheck, D., Weeks, J.W., Croft, C., Rybenkov, V.V., Zgurskaya, H.I., 2016. Breaking the permeability barrier of *Escherichia coli* by controlled hyperporination of the outer membrane. Antimicrobial agents and chemotherapy 60, 7372.

Kvist, T., Ahring, B.K., Westermann, P., 2007. Archaeal diversity in Icelandic hot springs. FEMS Microbiology Ecology 59, 71-80.

López-Lara, I.M., Gao, J.-L., Soto, M.J., Solares-Pérez, A., Weissenmayer, B., Sohlenkamp, C., Verroios, G.P., Thomas-Oates, J., Geiger, O., 2005. Phosphorus-free membrane lipids of *Sinorhizobium meliloti* are not required for the symbiosis with Alfalfa but contribute to increased cell yields under phosphorus-Limiting conditions of growth. Molecular Plant-Microbe Interactions 18, 973-982.

López-Lara, I.M., Geiger, O., 2001. Novel pathway for phosphatidylcholine biosynthesis in bacteria associated with eukaryotes. J Biotechnol 91, 211-221.

López-Lara, I.M., Sohlenkamp, C., Geiger, O., 2003. Membrane Lipids in Plant-Associated Bacteria: Their Biosyntheses and Possible Functions. Molecular Plant-Microbe Interactions 16, 567-579.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vazquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nat Commun 6.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806.

Lipp, J.S., Hinrichs, K.-U., 2009. Structural diversity and fate of intact polar lipids in marine sediments. Geochimica et Cosmochimica Acta 73, 6816-6833.

Lipp, J.S., Morono, Y., Inagaki, F., Hinrichs, K.-U., 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. Nature 454, 991-994.

Logemann, J., Graue, J., Köster, J., Engelen, B., Rullkötter, J., Cypionka, H., 2011. A laboratory experiment of intact polar lipid degradation in sandy sediments. Biogeosciences 8, 2547-2560.

Long, S.R., 1989. Rhizobium-legume nodulation: Life together in the underground. Cell 56, 203-214.

Loomis, S.E., Russell, J.M., Sinninghe Damsté, J.S., 2011. Distributions of branched GDGTs in soils and lake sediments from western Uganda: Implications for a lacustrine paleothermometer. Organic Geochemistry 42, 739-751.

Madigan, M.T., 1995. Microbiology of nitrogen fixation by anoxygenic photosynthetic bacteria, in: blankenship, R.E., Madigan, M.T., Bauer, C.E. (Eds.), Anoxygenic Photosynthetic Bacteria. Springer Netherlands, Dordrecht, pp. 915-928.

Madigan, M.T., Jung, D.O., 2009. An Overview of Purple Bacteria: Systematics, Physiology, and Habitats, in: Hunter, C.N., Daldal, F., Thurnauer, M.C., Beatty, J.T. (Eds.), The purple phototrophic bacteria. Springer Netherlands, Dordrecht, pp. 1-15.

Madsen, E.L., 2000. Nucleic-acid characterization of the identity and activity of subsurface microorganisms. Hydrogeology Journal 8, 112-125.

Martínez-Morales, F., Schobert, M., López-Lara, I.M., Geiger, O., 2003. Pathways for phosphatidylcholine biosynthesis in bacteria. Microbiology 149, 3461-3471.

Martens, R., 1995. Current methods for measuring microbial biomass C in soil: Potentials and limitations. Biology and Fertility of Soils 19, 87-99.

Martino, D.P., Grossman, E.L., Ulrich, G.A., Burger, K.C., Schlichenmeyer, J.L., Suflita, J.M., Ammerman, J.W., 1998. Microbial abundance and activity in a low-conductivity aquifer system in east-central Texas. Microbial Ecology 35, 224-234.

Masepohl, B., Drepper, T., Klipp, W., 2005. Nitrogen fixation in the photosynthetic purple bacterium *Rhodobacter capsulatus*, in: Klipp, W., Masepohl, B., Gallon, J.R., Newton, W.E. (Eds.), Genetics and regulation of nitrogen fixation in free-living bacteria. Springer Netherlands, Dordrecht, pp. 141-173.

Moser, R., Aktas, M., Narberhaus, F., 2014. Phosphatidylcholine biosynthesis in *Xanthomonas campestris* via a yeast-like acylation pathway. Molecular microbiology 91, 736-750.

Narasingarao, P., Podell, S., Ugalde, J.A., Brochier-Armanet, C., Emerson, J.B., Brocks, J.J., Heidelberg, K.B., Banfield, J.F., Allen, E.E., 2012. De novo metagenomic assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities. The ISME journal 6, 81-93.

Nikaido, H., Vaara, M., 1985. Molecular basis of bacterial outer membrane permeability. Microbiological reviews 49, 1-32.

Nowak, M.E., Schwab, V.F., Lazar, C.S., Behrendt, T., Kohlhepp, B., Totsche, K.U., Küsel, K., Trumbore, S.E., 2017. Carbon isotopes of dissolved inorganic carbon reflect utilization of different carbon sources by microbial communities in two limestone aquifer assemblages. Hydrol. Earth Syst. Sci. 21, 4283-4300.

Oelmann, Y., Kreutziger, Y., Temperton, V.M., Buchmann, N., Roscher, C., Schumacher, J., Schulze, E.-D., Weisser, W.W., Wilcke, W., 2007. Nitrogen and phosphorus budgets in experimental grasslands of variable diversity. Journal of Environmental Quality 36, 396-407.

Op den Kamp, J.A.F., 1979. Lipid asymmetry in membranes. Annual Review of Biochemistry 48, 47-71.

Ouverney, C.C., Fuhrman, J.A., 2000. Marine planktonic archaea take up amino acids. Applied and environmental microbiology 66, 4829-4833.

Pearson, A., 2014. 12.11 - Lipidomics for geochemistry, in: Holland, H.D., Turekian, K.K. (Eds.), Treatise on Geochemistry (Second Edition). Elsevier, Oxford, pp. 291-336.

Pereyra, M.A., Zalazar, C.A., Barassi, C.A., 2006. Root phospholipids in *Azospirillum*-inoculated wheat seedlings exposed to water stress. Plant Physiology and Biochemistry 44, 873-879.

Peterse, F., Hopmans, E.C., Schouten, S., Mets, A., Rijpstra, W.I.C., Sinninghe Damsté, J.S., 2011. Identification and distribution of intact polar branched tetraether lipids in peat and soil. Organic Geochemistry 42, 1007-1015.

Peterse, F., Kim, J.-H., Schouten, S., Kristensen, D.K., Koç, N., Sinninghe Damsté, J.S., 2009. Constraints on the application of the MBT/CBT palaeothermometer at high latitude environments (Svalbard, Norway). Organic Geochemistry 40, 692-699.

Peterse, F., van der Meer, J., Schouten, S., Weijers, J.W.H., Fierer, N., Jackson, R.B., Kim, J.-H., Sinninghe Damsté, J.S., 2012. Revised calibration of the MBT–CBT paleotemperature proxy based on branched tetraether membrane lipids in surface soils. Geochimica et Cosmochimica Acta 96, 215-229.

Pitcher, A., Hopmans, E.C., Mosier, A.C., Park, S.-J., Rhee, S.-K., Francis, C.A., Schouten, S., Sinninghe Damsté, J.S., 2011. Core and intact polar glycerol dibiphytanyl

glycerol tetraether lipids of ammonia-oxidizing archaea enriched from marine and estuarine sediments. Applied and Environmental Microbiology 77, 3468-3477.

Pitcher, A., Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2009. Separation of core and intact polar archaeal tetraether lipids using silica columns: Insights into living and fossil biomass contributions. Organic Geochemistry 40, 12-19.

Pitcher, A., Rychlik, N., Hopmans, E.C., Spieck, E., Rijpstra, W.I.C., Ossebaar, J., Schouten, S., Wagner, M., Sinninghe Damste, J.S., 2010. Crenarchaeol dominates the membrane lipids of *Candidatus Nitrososphaera* gargensis, a thermophilic Group I.1b Archaeon. ISME J 4, 542-552.

Powers, L., Werne, J.P., Vanderwoude, A.J., Sinninghe Damsté, J.S., Hopmans, E.C., Schouten, S., 2010. Applicability and calibration of the TEX86 paleothermometer in lakes. Organic Geochemistry 41, 404-413.

Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., Bakker, J.D., Cleland, E.E., DeCrappeo, N.M., DeLorenze, E., Hagenah, N., Hautier, Y., Hofmockel, K.S., Kirkman, K.P., Knops, J.M.H., La Pierre, K.J., MacDougall, A.S., McCulley, R.L., Mitchell, C.E., Risch, A.C., Schuetz, M., Stevens, C.J., Williams, R.J., Fierer, N., 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology Letters 18, 85-95.

Rütters, H., Sass, H., Cypionka, H., Rullkötter, J., 2002. Microbial communities in a Wadden Sea sediment core—clues from analyses of intact glyceride lipids, and released fatty acids. Organic Geochemistry 33, 803-816.

Raetz, C., Dowhan, W., 1990. Biosynthesis and function of phospholipids in Escherichia coli. Journal of Biological Chemistry 265, 1235-1238.

Rappé, M.S., Connon, S.A., Vergin, K.L., Giovannoni, S.J., 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418, 630.

Rethemeyer, J., Schubotz, F., Talbot, H.M., Cooke, M.P., Hinrichs, K.-U., Mollenhauer, G., 2010. Distribution of polar membrane lipids in permafrost soils and sediments of a small high Arctic catchment. Organic Geochemistry 41, 1130-1145.

Řezanka, T., Sigler, K., 2009. Odd-numbered very-long-chain fatty acids from the microbial, animal and plant kingdoms. Progress in Lipid Research 48, 206-238.

Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytologist 171, 41-53.

Romantsov, T., Guan, Z., Wood, J.M., 2009. Cardiolipin and the osmotic stress responses of bacteria. Biochimica et Biophysica Acta (BBA)-Biomembranes 1788, 2092-2100.

Rooney-Varga, J.N., Anderson, R.T., Fraga, J.L., Ringelberg, D., Lovley, D.R., 1999. Microbial communities associated with anaerobic benzene degradation in a petroleum-contaminated aquifer. Applied and Environmental Microbiology 65, 3056-3063.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W.W., Schmid, B., Schulze, E.-D., 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic and Applied Ecology 5, 107-121.

Russell, N.J., Evans, R.I., ter Steeg, P.F., Hellemons, J., Verheul, A., Abee, T., 1995. Membranes as a target for stress adaptation. International Journal of Food Microbiology 28, 255-261.

Salvagiotti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A., Dobermann, A., 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. Field Crops Research 108, 1-13.

Sato, N., 1992. Betaine lipids. The botanical magazine, 105, 185-197.

Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3, 348.

Schippers, A., Neretin, L.N., Kallmeyer, J., Ferdelman, T.G., Cragg, B.A., John Parkes, R., Jorgensen, B.B., 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. Nature 433, 861-864.

Schmidt, E., Paul, E., 1982. Microscopic methods for soil microorganisms. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 803-814.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Schoug, Å., Fischer, J., Heipieper, H.J., Schnürer, J., Håkansson, S., 2008. Impact of fermentation pH and temperature on freeze-drying survival and membrane lipid composition of Lactobacillus coryniformis Si3. Journal of Industrial Microbiology & Biotechnology 35, 175-181.

Schouten, S., Hopmans, E.C., Pancost, R.D., Damsté, J.S.S., 2000. Widespread occurrence of structurally diverse tetraether membrane lipids: Evidence for the ubiquitous presence of low-temperature relatives of hyperthermophiles. Proceedings of the National Academy of Sciences 97, 14421-14426.

Schouten, S., Hopmans, E.C., Schefuß, E., Sinninghe Damsté, J.S., 2002. Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures? Earth and Planetary Science Letters 204, 265-274.

Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. Organic Geochemistry 54, 19-61.

Schouten, S., Huguet, C., Hopmans, E.C., Kienhuis, M.V.M., Sinninghe Damsté, J.S., 2007. Analytical methodology for TEX86 paleothermometry by high-performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry. Analytical Chemistry 79, 2940-2944.

Schouten, S., Middelburg, J.J., Hopmans, E.C., Sinninghe Damsté, J.S., 2010. Fossilization and degradation of intact polar lipids in deep subsurface sediments: A theoretical approach. Geochimica et Cosmochimica Acta 74, 3806-3814.

Schouten, S., Pitcher, A., Hopmans, E.C., Villanueva, L., van Bleijswijk, J., Sinninghe Damsté, J.S., 2012. Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone: I. Selective preservation and degradation in the water column and consequences for the TEX86. Geochimica et Cosmochimica Acta 98, 228-243.

Schubotz, F., Wakeham, S.G., Lipp, J.S., Fredricks, H.F., Hinrichs, K.-U., 2009. Detection of microbial biomass by intact polar membrane lipid analysis in the water column and surface sediments of the Black Sea. Environmental Microbiology 11, 2720-2734.

Schwab, V.F., Hermann, M., Roth, V.N., Gleixner, G., Lehmann, R., Pohnert, G., Trumbore, S., Küsel, K., Totsche, K., 2016. Functional diversity of microbial communities in pristine aquifers inferred by PLFA - and sequencing - based approaches. Biogeosciences 2016, 1-45.

Schwab, V.F., Herrmann, M., Roth, V.N., Gleixner, G., Lehmann, R., Pohnert, G., Trumbore, S., Küsel, K., Totsche, K.U., 2017. Functional diversity of microbial communities in pristine aquifers inferred by PLFA- and sequencing-based approaches. Biogeosciences 14, 2697-2714.

Shadiac, N., Nagarajan, Y., Waters, S., Hrmova, M., 2013. Close allies in membrane protein research: Cell-free synthesis and nanotechnology. Molecular Membrane Biology 30, 229-245.

Shaw, N., 1974. Lipid composition as a guide to the classification of bacteria, in: Perlman, D. (Ed.), Advances in Applied Microbiology. Academic Press, pp. 63-108.

Shevchenko, A., Simons, K., 2010. Lipidomics: coming to grips with lipid diversity. Nat Rev Mol Cell Biol 11, 593-598.

Silhavy, T.J., Kahne, D., Walker, S., 2010. The bacterial cell envelope. Cold Spring Harbor perspectives in biology 2, 5.

Sinninghe Damsté, J.S., Hopmans, E.C., Pancost, R.D., Schouten, S., Geenevasen, J.A., 2000. Newly discovered non-isoprenoid glycerol dialkyl glycerol tetraether lipids in sediments. Chemical Communications, 1683-1684.

Sinninghe Damsté, J.S., Ossebaar, J., Schouten, S., Verschuren, D., 2008. Altitudinal shifts in the branched tetraether lipid distribution in soil from Mt. Kilimanjaro (Tanzania): Implications for the MBT/CBT continental palaeothermometer. Organic Geochemistry 39, 1072-1076.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Weijers, J.W.H., Foesel, B.U., Overmann, J., Dedysh, S.N., 2011. 13,16-dimethyl octacosanedioic acid (iso-

Diabolic Acid), a common membrane-spanning lipid of *Acidobacteria* Subdivisions 1 and 3. Applied and environmental microbiology 77, 4147-4154.

Sinninghe Damsté, J.S., Schouten, S., Hopmans, E.C., van Duin, A.C.T., Geenevasen, J.A.J., 2002. Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. Journal of Lipid Research 43, 1641-1651.

Smith, R.J., Jeffries, T.C., Roudnew, B., Fitch, A.J., Seymour, J.R., Delpin, M.W., Newton, K., Brown, M.H., Mitchell, J.G., 2012. Metagenomic comparison of microbial communities inhabiting confined and unconfined aquifer ecosystems. Environmental Microbiology 14, 240-253.

Sohlenkamp, C., Geiger, O., 2016. Bacterial membrane lipids: diversity in structures and pathways. FEMS Microbiology Reviews 40, 133-159.

Sohlenkamp, C., López-Lara, I.M., Geiger, O., 2003. Biosynthesis of phosphatidylcholine in bacteria. Progress in Lipid Research 42, 115-162.

Stewart, E.J., 2012. Growing unculturable bacteria. Journal of Bacteriology 194, 4151-4160.

Stouthamer, R., Luck, R., 1993. Influence of microbe-associated parthenogenesis on the fecundity of Trichogramma deion and T. pretiosum. Entomologia Experimentalis et Applicata 67, 183-192.

Strahl, H., Errington, J., 2017. Bacterial membranes: structure, domains, and function. Annual Review of Microbiology 71, 519-538.

Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology. Rapid Communications in Mass Spectrometry 18, 617-628.

Sundh, I., Börjesson, G., Tunlid, A., 2000. Methane oxidation and phospholipid fatty acid composition in a podzolic soil profile. Soil Biology and Biochemistry 32, 1025-1028.

Tai, S.-P., Hoger, J.H., Kaplan, S., 1986. Phospholipid transfer activity in synchronous populations of Rhodobacter sphaeroides. Biochimica et Biophysica Acta (BBA) - Biomembranes 859, 198-208.

Tateishi, T., Horikoshi, T., Tsubota, H., Takahashi, F., 1989. Application of the chloroform fumigation-incubation method to the estimation of soil microbial biomass in burned and unburned Japanese red pine forests. FEMS Microbiology Letters 62, 163-171.

Torsvik, V., Goksøyr, J., Daae, F.L., 1990. High diversity in DNA of soil bacteria. Applied and environmental microbiology 56, 782-787.

Turich, C., Freeman, K.H., Bruns, M.A., Conte, M., Jones, A.D., Wakeham, S.G., 2007. Lipids of marine archaea: Patterns and provenance in the water-column and sediments. Geochimica et Cosmochimica Acta 71, 3272-3291.

Uda, I., Sugai, A., Itoh, Y., Itoh, T., 2001. Variation in molecular species of polar lipids from Thermoplasma acidophilum depends on growth temperature. Lipids 36, 103-105.

Vadakattu, G., Paterson, J., 2006. Free-living bacteria lift soil nitrogen supply. Farming Ahead 169, 40.

van den Wollenberg, A., 1977. Redundancy analysis an alternative for canonical correlation analysis. Psychometrika 42, 207-219.

Van Der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2007. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296-310.

Van Mooy, B.A.S., Fredricks, H.F., Pedler, B.E., Dyhrman, S.T., Karl, D.M., Koblížek, M., Lomas, M.W., Mincer, T.J., Moore, L.R., Moutin, T., Rappé, M.S., Webb, E.A., 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature 458, 69.

Vences-Guzmán, M.Á., Geiger, O., Sohlenkamp, C., 2012. Ornithine lipids and their structural modifications: from A to E and beyond. FEMS Microbiology Letters 335, 1-10.

Wörmer, L., Lipp, J.S., Hinrichs, K.-U., 2015. Comprehensive analysis of microbial lipids in environmental samples through HPLC-MS protocols, in: McGenity, T.J.,

Timmis, K.N., Nogales, B. (Eds.), Hydrocarbon and Lipid Microbiology Protocols: Petroleum, Hydrocarbon and Lipid Analysis. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 289-317.

Wörmer, L., Lipp, J.S., Schröder, J.M., Hinrichs, K.-U., 2013. Application of two new LC–ESI–MS methods for improved detection of intact polar lipids (IPLs) in environmental samples. Organic Geochemistry 59, 10-21.

Wang, X., Li, W., Li, M., Welti, R., 2006. Profiling lipid changes in plant response to low temperatures. Physiologia Plantarum 126, 90-96.

Weber, Y., De Jonge, C., Rijpstra, W.I.C., Hopmans, E.C., Stadnitskaia, A., Schubert, C.J., Lehmann, M.F., Sinninghe Damsté, J.S., Niemann, H., 2015. Identification and carbon isotope composition of a novel branched GDGT isomer in lake sediments: Evidence for lacustrine branched GDGT production. Geochimica et Cosmochimica Acta 154, 118-129.

Weijers, J.W., Panoto, E., van Bleijswijk, J., Schouten, S., Rijpstra, W.I.C., Balk, M., Stams, A.J., Damste, J.S.S., 2009. Constraints on the biological source (s) of the orphan branched tetraether membrane lipids. Geomicrobiology Journal 26, 402-414.

Weijers, J.W., Schouten, S., Hopmans, E.C., Geenevasen, J.A., David, O.R., Coleman, J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006a. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. Environmental Microbiology 8, 648-657.

Weijers, J.W.H., Schouten, S., Hopmans, E.C., Geenevasen, J.A.J., David, O.R.P., Coleman, J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006b. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. Environmental Microbiology 8, 648-657.

Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007. Environmental controls on bacterial tetraether membrane lipid distribution in soils. Geochimica et Cosmochimica Acta 71, 703-713.

Weisser, W.W., Roscher, C., Meyer, S.T., Ebeling, A., Luo, G., Allan, E., Beßler, H., Barnard, R.L., Buchmann, N., Buscot, F., Engels, C., Fischer, C., Fischer, M.,

Gessler, A., Gleixner, G., Halle, S., Hildebrandt, A., Hillebrand, H., de Kroon, H., Lange, M., Leimer, S., Le Roux, X., Milcu, A., Mommer, L., Niklaus, P.A., Oelmann, Y., Proulx, R., Roy, J., Scherber, C., Scherer-Lorenzen, M., Scheu, S., Tscharntke, T., Wachendorf, M., Wagg, C., Weigelt, A., Wilcke, W., Wirth, C., Schulze, E.-D., Schmid, B., Eisenhauer, N., 2017. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. Basic and Applied Ecology 23, 1-73.

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H.-E., Rajashekar, C.B., Williams, T.D., Wang, X., 2002. Profiling membrane lipids in plant stress responses: role of phospholipase Dα in freezing-induced lipid changes in arabidopsis. Journal of Biological Chemistry 277, 31994-32002.

Welti, R., Wang, X., 2004. Lipid species profiling: a high-throughput approach to identify lipid compositional changes and determine the function of genes involved in lipid metabolism and signaling. Current Opinion in Plant Biology 7, 337-344.

Wenk, M.R., 2005. The emerging field of lipidomics. Nat Rev Drug Discov 4, 594-610.

Wetherell, D., Dougall, D., 1976. Sources of nitrogen supporting growth and embryogenesis in cultured wild carrot tissue. Physiologia Plantarum 37, 97-103.

White, D., Davis, W., Nickels, J., King, J., Bobbie, R., 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40, 51-62.

Woese, C.R., 1987. Bacterial evolution. Microbiological reviews 51, 221-271.

Woese, C.R., Fox, G.E., 1977. Phylogenetic structure of the prokaryotic domain: The primary kingdoms. Proceedings of the National Academy of Sciences 74, 5088-5090.

Woese, C.R., Kandler, O., Wheelis, M.L., 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proceedings of the National Academy of Sciences 87, 4576-4579.

Yang, H., Lü, X., Ding, W., Lei, Y., Dang, X., Xie, S., 2015. The 6-methyl branched tetraethers significantly affect the performance of the methylation index (MBT′) in

soils from an altitudinal transect at Mount Shennongjia. Organic Geochemistry 82, 42-53.

Yang, K., Han, X., 2016. Lipidomics: techniques, applications, and outcomes related to biomedical sciences. Trends in Biochemical Sciences 41, 954-969.

Zahran, H.H., 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Reviews 63, 968-989.

Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35, 275-294.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111-129.

Zengler, K., Toledo, G., Rappé, M., Elkins, J., Mathur, E.J., Short, J.M., Keller, M., 2002. Cultivating the uncultured. Proceedings of the National Academy of Sciences 99, 15681-15686.

Zhan, J., Sun, Q., 2012. Diversity of free-living nitrogen-fixing microorganisms in the rhizosphere and non-rhizosphere of pioneer plants growing on wastelands of copper mine tailings. Microbiological Research 167, 157-165.

Zhang, X., Ferguson-Miller, S.M., Reid, G.E., 2009. Characterization of ornithine and glutamine lipids extracted from cell membranes of *Rhodobacter sphaeroides*. Journal of the American Society for Mass Spectrometry 20, 198-212.

Zhang, Y.-M., Rock, C.O., 2008. Membrane lipid homeostasis in bacteria. Nature Reviews Microbiology 6, 222-233.

Zhou, Y., Kellermann, C., Griebler, C., 2012. Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer. FEMS Microbiology Ecology 81, 230-242.

Zinger, L., Gobet, A., Pommier, T., 2012. Two decades of describing the unseen majority of aquatic microbial diversity. Molecular Ecology 21, 1878-1896.

Zink, K.-G., Wilkes, H., Disko, U., Elvert, M., Horsfield, B., 2003. Intact phospholipids—microbial “life markers” in marine deep subsurface sediments. Organic Geochemistry 34, 755-769.

Curriculum Vitae

Su Ding

Selbständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen und Quellen angefertigt habe.

Ort, Datum, Unterschrift

Jena 31.01.2019

Su Ding